The Journal of Laboratory and Clinical Medicine

VOL. 26 NOVEMBER, 1940 No. 2

PROGRESS

TUBERCULOSIS—PRESENT AND FUTURE

GERALD B. WEBB, M.D., COLORADO SPRINGS, COLO.

IN MOST parts of the world where statistics have been kept the death rate from tuberculosis has been steadily declining for about one hundred years.

About 1780 the estimated death rate from consumption in England was 650 per 100,000 living inhabitants.

It is expected that the death rate in the United States will fall below 45 per 100,000 in 1940. According to Dublin, this will represent a decline of 37 per cent in the last ten years, and a decline of 61 per cent in the last twenty years. In forty years the death rate has dropped 76 per cent.

It is probable that tuberculosis has waxed and waned in different countries during the world's history. Historical studies suggest that an epidemiologic period may run over two hundred years.

In London there was a high death rate in 1650 which then declined and rose again to a high peak in 1780.

We are now approaching the lower level of a declining cycle and with the enlightenment which is ours today a new cycle should never begin. It is curious that in studying the present decline, which is common to most countries where statistics are available, that this decline has been almost continuous and that the discovery of the bacillus in 1882, the increasing isolation of the tuberculous in sanatoriums since 1900, and the increase in collapse therapy since 1912, have not definitely accelerated the decline.

In this country the problem is still a serious one among the colored population and among the Mexican residents. In spite of the excellent work of clinics and agencies, many "carriers" of the disease continue to be undiscovered.

Relapse is still too frequent and this is often the result of too short a sanatorium regime as well as lack of aftercare. The time will come when every open case of tuberculosis will be segregated. This will mean that many States must increase their sanatorium beds. Approximately 600,000 individuals in this country have active tuberculosis. Only 90,000 sanatorium beds are available when there should be 150,000.

Thanks to the Department of Agriculture bovine tuberculosis is being rapidly eliminated. The more general pasteurization of milk has greatly lessened human infection by the bovine bacillus.

Teaching the detection, control, and eradication of tuberculosis should begin in the public schools. Children should be taken into the fields to study Nature and to be shown the parasitic diseases of the flora and fauna. With such beginnings the problem of human disease will be more easily imparted to them.

The detection of childhood tuberculosis by the increasing application of the intradermal skin test with the purified protein derivative has been a great advance in preventive medicine. Reactors can be carefully watched and their young lives can be controlled. In many communities Nutrition Camps have been established, and these are ideal for children who are below par or who react to tuberculin.

More and more x-ray examinations of young adults and children are employed; these examinations should be repeated at intervals on any person suspected of having the disease. The use of miniature films in surveys of large groups is of increasing value.

Since clinical and biographical studies show tuberculosis is frequently a family disease, physicians who attend such families have great responsibility.

For many years the trend of treatment has been against climate and also against the long rest regime, although both factors have been of definite value.

Since 1912 various forms of collapse therapy have been introduced. Many physicians feel such therapy should be instituted as soon as the diagnosis of adult pulmonary tuberculosis has been made.

Added to artificial pneumothorax we have thoracoplasty, phrenic nerve crushing, extrapleural pneumothorax, and other surgical procedures which now include the Monaldi procedure of suction aspiration of tuberculous cavities. It is possible the pendulum has already swung too far in the surgical direction. Artificial pneumothorax is gradually giving way to more and more thoracoplastic operations. In spite of the help from pneumonolysis too many results are incomplete and too many complications still occur from pneumothorax treatment. The length of treatment is also a handicap.

While physical examination still plays an important part in diagnosis and study, the x-ray has largely supplanted this and is of great value in guiding pneumothorax treatment. Laminagraphy is now adding more important information.

The present sees an increase in the use of the bronchoscope for the diagnosis of bronchial lesions and their treatment, for the detection of strictures when weighing thoracoplasty, and for final proof that sputum is negative.

In research laboratories the chemistry of the tubercle bacillus is being carefully studied, and what is of probably more importance the growth factors are being discovered.

All efforts to discover a chemotherapy treatment have failed. Numerous laboratories have investigated the value of sulfanilamide, of sulfapyridine, and other derivatives of these drugs, but no success has been obtained.

The increase of blood platelets in active and chronic tuberculosis has never been explained by laboratory workers.

Clinicians note "leads" which may be followed. Why, in patients with diabetes, who become infected, does the tubercle bacillus progress so rapidly? Why do certain families, who are prone to high blood pressure and to nephritis, seem so immune to tubercle bacillus destruction?

Clinicians have observed that in tuberculous families the children who resemble in constitution a parent who has belonged to the high blood pressure group have escaped serious infection when the children of the tuberculous parent's type died.

Why is there a lessened incidence of pulmonary tuberculosis in patients who develop hyperthyroidism?

Vaccination by B.C.G. (Bacillus Calmette Guerin) has gained in usage in Europe, and laboratories in this country have established some value in animals, especially with intravenous administration.

With the continued decline in the death rate a form of vaccination may not be necessary. Shortage of food may again increase the number of tuberculous victims on the continent of Europe. The improved housing and living conditions in the United States, together with the activities of the National and State Tuberculosis Associations, should assuredly prevent another serious rise in the tuberculosis death rate in this country.

CLINICAL AND EXPERIMENTAL

DISSECTING ANEURYSM OF THE AORTA*

Case Report

Abel Levitt, M.D., F.A.C.P., Dexter S. Levy, B.A., M.D., and James R. L. Cole, M.D., Buffalo, N. Y.

THE relative infrequency with which the diagnosis of dissecting aneurysm of the aorta is made ante mortem has prompted us to report a case observed on the wards of the Edward J. Meyer Memorial Hospital. For many years this disease has been known to pathologists, but until recently the clinical observations have been few and the diagnosis has been made at post mortem. The true explanation of this probably lies in the fact that the disease is often confused with various intrathoracic or intra-abdominal conditions which it closely simulates. The relative infrequency of the ante-mortem diagnosis is more evident when it is realized that only twenty-five cases can be found reported in the literature.[1] The disease itself occurs predominantly in males between the ages of 40 and 60 years, in whom there is a usual history of hypertension. There is no pattern to be followed in the diagnosis, but certain features when present must be analyzed in proper combinations to eventuate the correct diagnosis.[2] These include: (1) the sudden onset of pain in a person with hypertension. This pain not infrequently radiates to other portions of the body but rarely the arms; (2) the presence of a rapid, enlarged heart with or without murmurs; (3) the presence of shock; (4) slight fever and leucocytosis; (5) dyspnea and cyanosis; (6) variation in the pulses between the upper and lower extremities.

There have been many theories advanced as to the cause of the disease, and the division into predisposing and exciting factors has been advocated.[3] The predisposing factors include:[4] (1) Virchow's view that most of these aneurysms arise on the basis of atheromatous ulcers. The fact that arteriosclerosis was not observed in some of the cases at necropsy makes this a tenable explanation in only a few. (2) Inflammation which may weaken the wall of the aorta sufficiently to predispose it to rupture. (3) Medial degeneration of the aorta, in which there is focal hyaline degeneration resulting in cyst formation, has recently been recognized as strongly predisposing to the disease.

With the presence of a predisposing factor, however, the problem of what is the actual causative factor presents itself. Here it has been agreed that two primary factors play a part: (1) physical trauma, and (2) internal trauma due to either emotion or exertion.

*From the Medical Service of the Edward J. Meyer Memorial Hospital (Buffalo City Hospital) and the School of Medicine, University of Buffalo.
Received for publication, October 16, 1939.

Pathologically[5] the aorta usually shows an irregular tear which extends through the interior and partly through the media into which blood penetrates until it finds a plane of cleavage and begins to dissect. The blood enclosed in this sheath composed only of adventitia and media most often perforates at some point.

Clinically, as already indicated, certain features suggest the diagnosis. The patients are acutely ill and fail progressively, and the ultimate prognosis is poor, with death occurring in from a few hours to several days. In the differential diagnosis (1) coronary thrombosis, (2) embolism, (3) abdominal catastrophe, and (4) pneumonia are most frequently confused with the diagnosis.

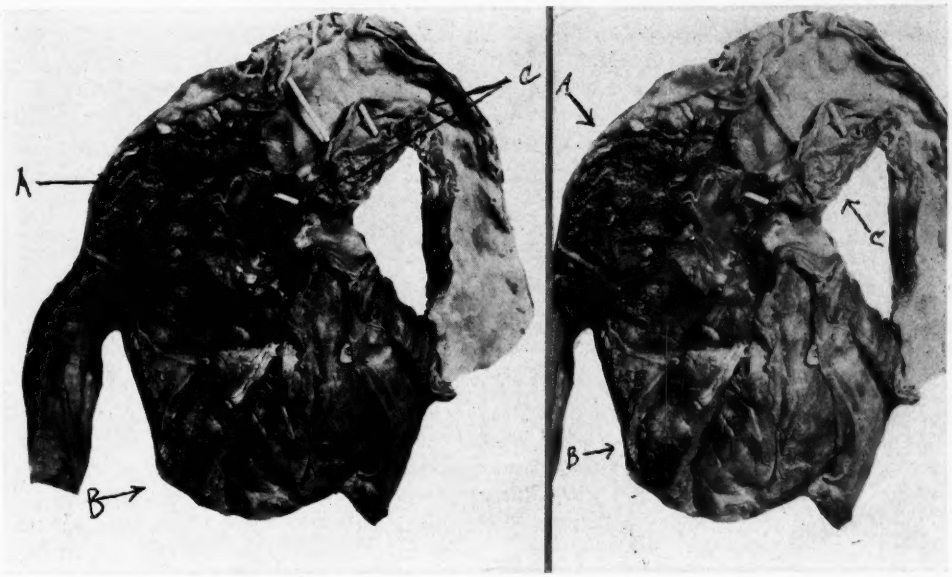


Fig. 1.—*A*, The large oval represents the hemorrhagic mass over the right auricle. It extends around the base of the heart and aorta with compression; *B*, the fibrous tag on the pericardium where a similar large amount of clot was found partly organized; *C*, the dissecting hemorrhage above, in between the coats, and below outside of aorta continuous with hemorrhage *A*.

CASE REPORT

CASE 379362.—The patient was a white male, aged 51 years, whose occupation was a fireman. He was referred to the hospital on December 5, 1938, with a history of eight hours' duration. He complained of being awakened from his sleep by a sharp, excruciating pain, located diffusely throughout the lower abdomen, but more pronounced about the umbilicus. This pain radiated through to the back, and at the onset was accompanied by emesis on two occasions. No blood was present in the emesis, and the stools were normal in color and consistency. His past history revealed that he first sought medical attention in January, 1937, at which time he was admitted to the diagnostic clinic. His history at that time was of precordial pain of three years' duration brought on by exercise. This pain occurred about one to two times monthly; it lasted one to two minutes and was accompanied by dyspnea, palpitation, and weakness. He also gave some history of occasional frontal headaches, poor vision, and nocturia two to five times. He denied past illness, except for urethritis at the age of 28. At this time (January, 1937) his physical findings revealed the following positive features:

Eyes: Pupils equal, regular, and reacted to light and accommodation.
Mouth: Dental caries.

Chest: Resonant and clear throughout.
Heart: Enlarged, regular, good tone, precordial systolic bruit, aortic second sound accentuated.
Blood pressure: 220/120—Vessels moderately firm.
Abdomen: Negative except for a small left inguinal hernia.
Extremities: No edema—reflexes normal.

His Wassermann test was negative and his electrocardiogram showed an occasional extraventricular systole with slurring QRS. He was advised to return for further therapy but failed to do so. The next association with him was at the time of his present admission. When admitted, he appeared to be well developed and well nourished but seemed to be suffering much pain. He was breathing about thirty times per minute but showed no cyanosis. The positive findings were as follows:

Chest: Elliptical in shape. Expansion somewhat limited on the right side. Resonance was unimpaired. The breath sounds were somewhat suppressed in the right base, otherwise vesicular throughout. No râles were heard.
Heart: Enlarged to the left, regular in rate, good tone. Aortic second sound accentuated. Blood pressure 220/124.
Abdomen: Moved with respiration. There was resistance to palpation in the right upper quadrant. Tenderness was present throughout, more so in the gall bladder region. No organs or masses were felt.
Extremities: No edema.

The impression at the time of admission was (1) hypertension with myocardial hypertrophy and dilatation, (2) possible cardiovascular syphilis and aneurysm, and (3) possible surgical abdomen.

Because of the possibility of an acute abdomen, surgical consultation was obtained. In addition to the findings already mentioned, the patient was now complaining of hemoptysis and pain in the chest on deep inspiration. His urine was negative and his white blood cell count was 22,250, with 92 per cent polymorphonuclear cells. The possibilities entertained at this time were (1) perforated ulcer, (2) coronary thrombosis, and (3) acute pancreatitis.

While no definite diagnosis was made, it was thought advisable to observe the patient on the surgical service. Approximately six hours after his transfer to the surgical service, we again saw him. The pain was still present in the abdomen, and the cough, productive of bright blood, persisted. The temperature which was 97.2° F. on admission was now 101.2° F., and the pulse had become more rapid, 88 to 136. The blood pressure which had been 200/104 on admission was now 140/92 on the left arm, and 170/100 on the right arm. His physical findings at this time showed:

Chest: Marked diminution of breath sounds in the right base.
Heart: Enlarged, regular in rate, tones moderately suppressed, a "G" string type of pericardial friction rub was heard in the fifth interspace to the left of the sternum.
Abdomen: There were fullness and spasm with definite tenderness in the right upper quadrant. No masses were palpable.
Extremities: Slight cyanosis of the nails.

The impression at this time was possible coronary thrombosis or dissecting aneurysm.

An electrocardiogram was taken which revealed left axis deviation and myocardial damage. X-ray at this time showed an enlarged heart with the aorta normal. Therapy consisted of opium sedation, rest, and observation. X-ray was repeated on December 20, 1938, and the heart showed an increase in diameter of fully 4 cm. On December 30 fluid was present in the left pleural space, and thoracentesis was performed with the removal of 300 c.c. of straw-colored fluid which microscopically revealed red blood cells, some granular debris, some polymorphonuclear cells with a predominant small round cell element. Although his treatment was symptomatic, the patient did not respond and death occurred on January 11, 1939.

Post-Mortem Findings.—The left pleural cavity contained 1,400 c.c. of a rather thin, clear, yellowish-brown exudate. This contained numerous clots and fibrinous tags, composed of yellowish-green material. The lungs were dark slate gray in color, with a black overmottling of anthracotic pigment. There were patchy areas where the lung was collapsed; these were firm on section and dark hemorrhagic in color. The parietal and visceral pleurae of the left side were fibrously thickened. Crepitation was present throughout both lungs. The cut surface was dark purplish red color and on compression oozed a large amount of serosanguineous frothy fluid. The lymph nodes at the hilus of the lung were enlarged and on section were black in color. The trachea-bronchial tree showed the mucous membrane covered with a marked amount of mucus.

Heart: The heart was markedly enlarged. The pericardium was adherent. On section through into the heart, a large, currant jellylike clot about the size of an orange, was found anterior to and somewhat to the right of the right ventricle in the pericardial space. This bulged considerable, making the heart appear large laterally. The aorta was split into two layers, this split extending to the base of the heart where the pericardium was attached around the aorta. There was a large amount of dark clotted blood between these layers. This space communicated with the large pericardial space filled with blood. The myocardium was of fair tone. The valves and valve orifices were clear. The septum left ventricle beneath the cusps revealed subendocardial hemorrhage. The aorta showed numerous thickened areas of raised yellowish plaques. One of these communicated with the split space. The pulmonary arteries and veins were clear.

Abdomen: Abdominal viscera were essentially normal.

Anatomic Diagnosis.—Aortal arteriosclerosis with dissection between the coats and rupturing into a pericardial pocket, a space otherwise obliterated by fibrous adhesion, was found.

CONCLUSION

1. A dissecting aneurysm of the aorta, proved anatomically, has been presented.
2. The difficulty of clinical diagnosis is emphasized.

REFERENCES

1. Claiborne, T. S., and Haller, Emory D.: Dissecting Aneurysm of the Aorta: Antemortem Diagnosis and Course of Fifty-Three Days. Case Report, Am. Heart J. **15**: 358, 1938.
2. Hamburger, Morton, Jr., and Ferris, Eugene B., Jr.: Dissecting Aneurysm. A Study of Six Recent Cases, Am. Heart J. **16**: 1, 1938.
3. Tyson, D. Dawson: Dissecting Aneurysms, Am. J. Path. **7**: 581, 1931.
4. Glendy, R. E., and Castleman, B., and White, P. D.: Dissecting Aneurysm of the Aorta, Am. Heart J. **13**: 129, 1937.
5. Boyd, William: Textbook of Pathology, Philadelphia, 1938, Lea & Febiger, p. 445.

A NOTE ON SACCHAROMYCES FRAGILIS JORGENSEN ASSOCIATED WITH PATHOLOGIC CONDITIONS IN HUMAN BEINGS*

NORMAN CALLENDER LAFFER, PH.D., TUCSON, ARIZ.

SACCHAROMYCES FRAGILIS was first isolated by Jorgensen from kefir, and has repeatedly been found in dairy products. Within the last fifteen years, there have been two reports of the isolation of yeastlike fungi from pathologic conditions in human beings in which the organisms proved to be *Saccharomyces fragilis* Jorgensen. In both cases, the organisms were first classified as some other species, but upon detailed study at the Centraalbureau voor Schimmelcultures at Baarn, Holland, they proved to be strains of *Saccharomyces fragilis*. In the first case, Redaelli in 1925 (Stelling-Dekker, 1931) reported the isolation of *Saccharomyces cavernicula* n. sp. from the lung of a tuberculous patient. Of this culture, Stelling-Dekker (1931) writes as follows: "A careful consideration of this species allows one to conclude that all major characteristics agree with those of *Saccharomyces fragilis* Jorgensen. The agreement is so definite that a separation of *Saccharomyces cavernicula* as a variety of *Saccharomyces fragilis* is not warranted. . . ." Another place she writes: "*Saccharomyces cavernicula* Redaelli is identical with *Saccharomyces fragilis* Jorgensen."

In 1935 Ciferri and Redaelli (Diddens and Lodder, 1939) described a species that had been isolated by Carco at Catania from a lesion of the human tonsils and pharynx. It was classified as *Monilia pinoyisimilis* (A. Castellani) Redaelli and Ciferri variety *Citelliana* Redaelli and Ciferri. Of this organism Diddens and Lodder (1939) write: "It evidently belongs to the genus *Saccharomyces* and it is completely identical with *Sacch. fragilis* Jorgensen."

The purpose of this article is to report an organism isolated by Miller (1935) and classified by him as *Monilia pseudotropicalis* Castellani. I secured a transfer of this strain and continued its study and found it to be identical with *Saccharomyces fragilis* Jorgensen (Laffer, 1936). Such results are not surprising for two reasons: first, the difficulty with which some strains are stimulated to produce ascospores; and second, the following statement by Diddens and Lodder (1939): "*Monilia pseudotropicalis* Castellani—*Candida pseudotropicalis* (Castellani) Basgal has to be considered as the imperfect stage of *Saccharomyces fragilis* Jorgenson." The material contained in the following paragraph is taken from the unpublished thesis of Miller (1935).†

Case history. The patient, a male, came to the physician with an acute infection of the tonsils, characterized by a sore throat, fever, malaise, anorexia, fetid breath, terribly coated tongue, headache, backache, and pains in the limbs. White or yellow patches of exudate were present in follicular form. Intensive treatment for follicular tonsillitis in the first seven

*From the Department of Bacteriology, University of Arizona, Tucson.
Received for publication, October 23, 1939.
†Published with the permission of Dr. Miller.

days did not show the slightest improvement. On the eighth day he was given 10,000 units of diphtheria antitoxin, and the dose was repeated on the ninth day; he recovered rapidly without further treatment. At no time were there objective signs of diphtheria.

According to Miller, his culture isolated from this case had the characteristic fermentative reactions of *Monilia pseudotropicalis* Castellani and serologic studies confirmed this relationship. When the unknown strain was used to produce an antiserum, the antiserum reacted with its specific antigen 1:640; with one type culture of *Monilia pseudotropicalis* 1:640; and with a second type culture of *Monilia pseudotropicalis* 1:320. Agglutinin absorption tests substantiated these serologic findings. The usual morphologic studies were not reported in the thesis. In his conclusions Miller stated: "Culture 10Y which produced acid and gas in dextrose, levulose, galactose, sucrose, raffinose lactose, and inulin, corresponds almost exactly to two cultures of *Monilia pseudotropicalis* Castellani (one from New Orleans, Louisiana, and the other from London, England) which fermented the same carbohydrates with acid and gas. These three cultures formed a distinct group by agglutinin absorption."

In my study (Laffer, 1936) of this culture (Miller's 10Y) the cells were characterized as "cells oval and rod shaped, but becoming filamentous in older broth cultures and potato broth, and producing rod shaped to filamentous asci and ovoid to kidney shaped ascospores." The culture produces one to four ascospores per ascus when grown on carrot blocks or on carrot infusion agar for three days or more at room temperature. Careful examination of colonies on malt extract agar (Difco) streak plates showed pseudomycelia penetrating the substrate, with the groupings of the blastospores at the junction of the adjoining elements similar to those produced by the control culture of *Saccharomyces fragilis* Jorgensen, obtained from the Lister Institute (their No. 2303). Cells grown in malt extract broth (Difco) for twenty-four hours were more or less rod-shaped and measured 3.0 to 5.0 microns by 6.0 to 10.0 microns, with a few filamentous cells as long as 12.0 to 21.0 microns. In older cultures the length and number of the filamentous cells increased.

The biochemical changes produced by the unknown and the control culture were the same, namely: acid and gas in glucose, fructose, galactose, mannose, lactose, and inulin; acid and no gas in raffinose, and neither acid nor gas in arabinose, xylose, rhamnose, maltose, salicin, glycerol, mannitol, dextrin, and starch. For these determinations, nutrient broth base (Difco) plus 1 per cent carbohydrate and bromcresol purple was used. Litmus milk, if incubated at room temperature, became acid with evidence of gas production after a few days, but if incubated at 37° C., the milk was curdled with a renninlike curd. There was no evidence of proteolysis at the end of forty-five days of incubation. When tested by the Hammer and Collins (1935) method, the organism did not hydrolyze fat. No true liquefaction of gelatin was observed at 37° C. after forty-five days of incubation, while "softening" occurred in those cultures incubated at 18° C. to 25° C. for forty-two days. An uninoculated control showed no "softening." The term "softening" is used to indicate a stage in which the medium is soft at room temperature but the gelatin becomes solid upon cooling to 5° C. to 8° C. This term is used in contrast to true liquefaction in

which the medium fails to solidify upon cooling to the above temperatures. While Stelling-Dekker reports that *Saccharomyces fragilis* liquefies gelatin, a difference in the composition of the medium may account for the variation in the degree of utilization of the substrate.

SUMMARY

1. A third case in which *Saccharomyces fragilis* Jorgensen has been isolated from a pathologic condition in human beings is reported.

2. Confirmation of the relationship of *Saccharomyces fragilis* Jorgensen and *Candida* (Monilia) *pseudotropicalis* (Castellani) Basgal, as indicated by Diddens and Lodder (1939), is implied.

3. The desirability of an attempt to further confirm this relationship by serologic studies is suggested.

4. While nothing is contained to indicate that *Saccharomyces fragilis* is the causal agent in the infections of the throat, the finding of this species in throat cultures might prove worthy of a more detailed investigation or might prove to be a lead worthy of following. Careful morphologic studies of yeasts found, including repeated attempts at ascospore determination, and the inoculation of lactose, sucrose, inulin, maltose, litmus milk, and gelatin, would probably suffice for preliminary study and tentative identification.

The wide distribution of this organism is clearly shown. I have found this species in cream obtained in Illinois, and have identified as *Saccharomyces fragilis* cultures received from S. Orla-Jensen, Copenhagen, Denmark (from milk); from the late Dr. Henneberg, Kiel, Germany (from yoghurt); from W. Dorner, Liebefeld-Berne, Switzerland (from sour milk and whey); and from Erivan, Armenia, U. S. S. R. (from matzoon). It is easy to see how such organisms could get into the throat and thus be found in examination of throat cultures.

REFERENCES

Diddens, H. A., and Lodder, J.: On Some Sporogenous Yeasts and Their Imperfect Stages, Mycopathologia **2**: 28, 1939.

Hammer, B. W., and Colliers, M. A.: The Numbers of Lipolytic Bacteria in Various Dairy Products as Determined With Nile Blue Sulfate, Iowa Agricultural Experiment Station Research Bull. No. 169, pp. 73-92, 1934.

Laffer, N. C.: A Study of Lactose-fermenting Yeasts. Doctor's Dissertation, University of Illinois, Urbana, Illinois, 1936.

Miller, W. A.: A Study of Pathogenic Yeasts. Doctor's Dissertation, University of Illinois, Urbana, Illinois, 1935.

Stelling-Dekker, N. M.: Die Hefesammlung des Centraalbureau voor Schimmelcultures. Erster Teil. "Die Sporogenen Hefen," Verh. Kon. Akad. v. Wetensch. **28**: 1931.

THE ACTION OF EPINEPHRINE INJECTED INTO THE PERICARDIAL SAC*

PHILIP BLICKENSDORFER, PH.D., AND J. A. BONE, M.S., M.D.
CHICAGO, ILL.

WHILE the effect of epinephrine on blood pressure when given intravenously has been widely studied, the effect when given by other routes has not received adequate attention. When given by oral administration, it is usually inactive in normal people. Menninger[1] studied the effect of oral administration in various types of hyperthyroid cases. He gave the patients from 2 to 5 mg. of epinephrine in gelatin capsules and concluded that in some cases epinephrine may produce a marked effect on the circulation with rise of pressure. The effect results from absorption through the gastrointestinal tract and not from the mouth or throat.

Meltzer and Auer[2] found that the subcutaneous injection of 0.4 to 0.5 c.c. per kilogram of epinephrine has no influence on the blood pressure of a rabbit. Intraperitoneal injection also has no effect on the blood pressure, while 0.3 to 0.4 c.c. given intramuscularly may produce a rise of 40 to 50 mm. The rise is not so sudden as it is after intravenous injection.

Amberg[3] previously reported that doses of epinephrine that are fatal when given intravenously do not exert pressor effects in cats, dogs, or rabbits when given subcutaneously, although a very large dose (37 mg. per kilogram) raises the blood pressure in the dog.

Halsey[4] found that 27 mg. of epinephrine given intramuscularly to a 14 kg. dog raised the pressure 30 mm. (from 140 to 170). In man he found that the oral administration of epinephrine does not change the heart action or blood pressure; when subcutaneously administered, a dose of 0.5 to 1.5 mg., or about 0.5 to 1.5 c.c. of 1:1,000 solution, is uncertain in its effects on the blood pressure but usually causes a slight rise. This same dose, however, given intramuscularly may cause alarming symptoms and a rise of pressure of 90 mm. (110 to over 200). Since some people are sensitive to epinephrine, Halsey recommends that smaller doses be given until the reaction is known.

Grollman[5] quotes Euler and Liljestrand as having found that the subcutaneous injection of 0.7 mg. of epinephrine in man raised the blood pressure 17 mm. and increased the cardiac output from 4.3 liters to 7.5 liters per minute; this indicated both stimulation of cardiac activity and diminished peripheral resistance.

*From the Department of Pharmacology and Therapeutics, University of Illinois College of Medicine, Chicago.

Received for publication, November 1, 1939.

Auer and Gates[6] found that when 0.25 c.c. of epinephrine solution (1:1,000) per kilogram is given intratracheally in rabbits, a rise of pressure occurs after a few seconds and lasts for about five minutes. Jackson[7] records similar findings. We have found similar results to follow injection into the pericardial sac of dogs (see Figs. 1 and 2).

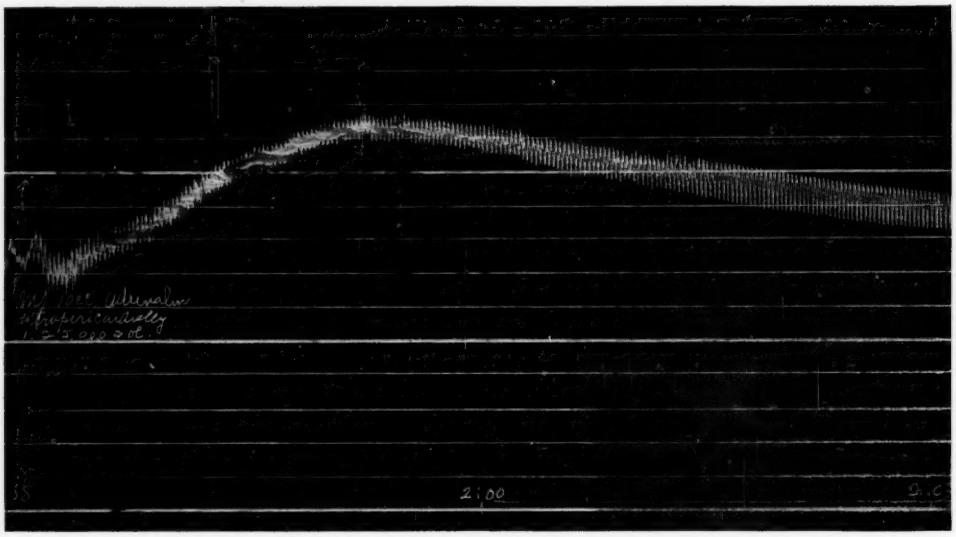


Fig. 1.—The effect of 10 c.c. epinephrine, 1:25,000, in the pericardial sac. (Dog—19 kg.)

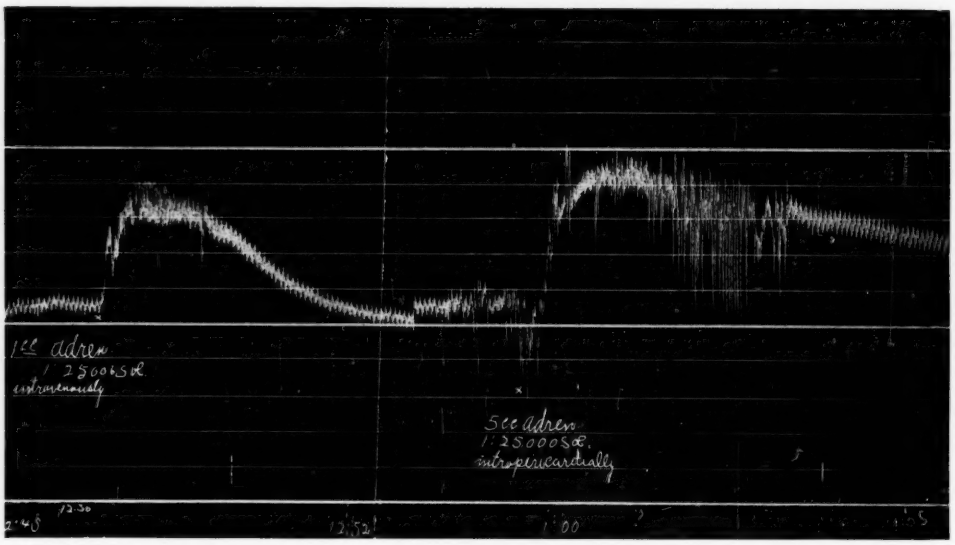


Fig. 2.—The comparative effect on blood pressure of 1 c.c. epinephrine, 1:25,000, intravenously and intrapericardially. The rapid rise in this case after intrapericardial injection is unusual.

METHOD

Dogs were anesthetized with 35 mg. per kilogram of pentobarbital in solution, given intraperitoneally. Blood pressure was recorded from the carotid artery. Artificial respiration was used throughout. The chest was opened and the solution of epinephrine placed in the pericardial sac so that none was lost

by leakage. Duodenal activity was recorded by the balloon method. The tracings show the effect. After and before injection into the pericardial sac, an injection was made into the saphenous vein in order to compare the relative effects of adrenalin when given intrapericardially and intravenously.

CONCLUSIONS

1. The effect of epinephrine injected into the pericardial sac is less abrupt but prolonged about five times as compared to the intravenous injection. About five to ten times the intravenous dose is necessary to produce identical rise in blood pressure.

2. The intrapericardial action of epinephrine in smaller doses is somewhat irregular on the blood pressure but is very constant on the intestines.

REFERENCES

1. Menninger, W. C.: Epinephrine, J. A. M. A. **84**: 1101, 1925.
2. Meltzer, S. J., and Auer, J.: The Rate of Absorption From Intramuscular Tissue, J. Exper. Med. **7**: 59, 1905.
3. Amberg, S.: Toxicität des Wirksamen Princips der Nebennieren, Arch. internat. de pharmacodyn. et de thérap. **11**: 57, 1902.
4. Halsey, J. I.: Observations on Adrenalin, Tr. A. Am. Physicians **24**: 520, 1909.
5. Grollman, A.: The Adrenals, Baltimore, 1936, Williams & Wilkins Co., p. 121.
6. Auer, J., and Gates, F. L.: Some Effects of Adrenalin When Injected Into the Respiratory Tract of Rabbits, J. Pharmacol. & Exper. Therap. **6**: 608, 1915.
7. Jackson, D.: Experimental Pharmacology, St. Louis, 1939, The C. V. Mosby Co., p. 142.

TORULA INFECTION OF THE CENTRAL NERVOUS SYSTEM*

A Report of a Case With Necropsy Findings

Leon H. Goldberg, M.D., Nyack, N. Y.

SINCE 1907 sporadic reports of torula infection have appeared in the literature. By far the most comprehensive monograph on the subject was presented by Stoddard and Cutler in 1916. In view of the frequency with which this infection is being reported, it is noteworthy to quote verbatim Stoddard's memorandum in 1916: "It would not have been too venturesome to have prophesied that the organism would be found at some future date producing nervous diseases in man. It will take time to determine how common this type of infection is, but many facts indicate the possibility of it being frequently present without recognition."

In 1939 McGrauder published protocols of 3 cases observed at the University of Virginia Hospital since October, 1935, in which he succeeded in isolating the organism. In a personal communication he submitted the following outline:

1. The technician who examines the spinal fluid must be torula conscious.

2. Identify as a yeast (as contrasted with cells) in the counting chamber. The count is made as usual with acetic acid to lake any red blood cells present.

*From the Medical Service, Nyack Hospital.
Received for publication, October 28, 1939.

Torulae might be mistaken for lymphocytes (and usually are) unless one has the probability of torula in mind. The torula organism will show some budding forms, and a thick wall, often surrounded by a definite capsule. No cytoplasm is seen.

3. If torula is suspected, mix a drop of sediment (from centrifuged spinal fluid) with a drop of Greenthal's stain on a slide, cover with a cover glass and examine. The stain will show definitely the nuclei and cytoplasm of the usual cells, distinguishing them clearly from the torulae, which show a definite "cellulose-like" wall, surrounded by a wide purplish or bluish capsule.

4. Confirm by culture. Torula usually reproduces by budding only, never forming mycelium, and does not ferment sugar.

It becomes evident that the apparent increase in reported cases is not necessarily an indication of an increased frequency of the infection in man, but rather an indication of an increased interest in the problem with a concurrent improvement in laboratory diagnosis.

The following case report, with its autopsy findings, is as typical of the vagaries of this infection as other cases reported to date.

CASE REPORT

A colored girl, aged 19 years, was first seen on March 3, 1938. She was prostrated, complained of an intractable headache of several weeks' duration, and had photophobia. The examination revealed a marked nuchal rigidity as the only positive sign of any cranial involvement. Ordinary palliative measures were of no avail. After three days, a spinal tap was done for diagnostic purposes as the picture suggested tuberculous meningitis. For the next three days there was no relief. On the assumption that it might be tuberculous meningitis, notwithstanding the negative findings in the spinal fluid, she was placed on a high vitamin D intake, and to my amazement she began to improve almost immediately. She returned to normal activity in two weeks.

Five months later the girl again became ill. In the early days of this recurrence, the picture was identical with that originally presented. This time, however, a series of neurologic signs appeared, following one another in rapid succession. On August 11 she lay in bed in a stupor, with her head and eyes sharply deviated to the right, while the right arm and leg were flaccid. The left hand constantly clutched at the bedclothes. The abdominal reflexes were absent. Both pupils reacted very sluggishly, and the right one was smaller than the left. There were reddening of the fundi, with engorgement of the veins. The jaw was tonically closed. There were positive Brudzinski and bilateral Hoffman signs. The following morning the patient was still in coma. The right limbs remained flaccid and both ankles showed clonus. Her temperature rose to 106° F.; her pulse rate increased to 140 and her respiratory rate remained between 20 and 30. On August 13 she died.

Her skin was dry and cool. Her heart, lungs, and abdomen were essentially negative. Neurologically, she continued to present a continually changing picture with such signs as positive Babinski on the right side and negative Babinski on the left. All four extremities were stiff and spastic. She moved the left side involuntarily but not the right side. The pupils reacted slug-

gishly; the veins dilated to twice the normal caliber. No diagnosis was made, although the usual series of differential diagnoses presented themselves with cerebral abscess as the most likely process.

Spinal Fluid Findings.—August 11, clear, under moderate pressure; cell count, zero; chlorides, 330 mg. per 100 c.c.; glucose, 10 mg. per 100 c.c.; smear lymphocytes, 2; tubercle bacilli, negative; Wassermann, negative; culture, negative. August 13, cell count, polymorphonuclears, 11, lymphocytes, 89; protein, 96; globulin, 2 plus; sugar, 31 mg. per 100 c.c.; chlorides, 715 mg. per 100 c.c.; colloidal gold sol, 1111233100; Wassermann, negative.

Necropsy Findings.—An autopsy was performed soon after death. The brain was examined by the Department of Pathology of the Neurological Institute. The skull was of normal thickness. The dura was moderately adherent to the inner surface of the skull. The brain weighed 1,300 Gm. At the base of the brain, in the interpeduncular fossa, on the inferior surface of the hypothalamus and about the optic chiasm and nerves, the leptomeninges were thickened and grayish with an accumulation of yellowish-gray exudate. On section of the cerebrum, no gross lesions of the parenchyma were noted. The spinal cord leptomeningeal blood vessels were congested, but otherwise the cord appeared normal.

Microscopic examination of the area revealed a proliferation of leptomeningeal cells in the form of fibroblasts. There was a heavy infiltration of the membrane by lymphocytes, monocytes, and plasma cells. There were numerous multinucleated giant cells about focal areas of necrosis.

In places in the necrotic areas, there were pale, translucent, round bodies, with double refractive margins. No structural details could be made out within them; they varied in size from that of lymphocytes to that of monocytes. Some of these bodies were also found within the giant cells. These proved to be *Torula histolytica*. There were also a terminal lobular pneumonia and acute ulceration of the duodenum.

CONCLUSION

A case with necropsy findings of *Torula histolytica* infection of the central nervous system is presented.

REFERENCES

1. Stoddard, James L., and Cutler, Elliot C.: Monographs of the Rockefeller Institute for Medical Research **6**: 1916.
2. Shapiro, Louis L., and Neal, Josephine B.: Torula Meningitis, Arch. Neurol. & Psychiat. **13**: 174, 1925.
3. Firor, W. M.: Torula Infection of the Central Nervous System, Internat. S. Digest **2**: 3.
4. Wortis, S. Bernard, and Wightman, Henry B.: A Case of Torula Meningitis, Bull. New York Acad. Med. **4**: 531, 1928.
5. Stone, Willard J., and Sturdivant, B. F.: Meningo-Encephalitis, Arch. Int. Med. **44**: 560, 1929.
6. Freeman, Walter: Torula Infection of the Central Nervous System, J. F. Psychol. u. Neurol. **43**: 236, 1931.
7. Johns, F. M., and Attaway, C. L.: Torula Meningitis, Am. J. Clin. Path. **3**: 459, 1933.
8. Levin, E. A.: Torula Infection of the Central Nervous System, Arch. Int. Med. **59**: 667, 1937.
9. Greenfield, J. G., Martin, J. P., and Moore, M. T.: Meningo-encephalitis Due to Cryptococcus meningitidis (Torula Histolytica), Lancet **2**: 1154, 1938.
10. Magrauder, R. Gregory: A Report of Three Cases of Torula Infection of the Central Nervous System, J. Lab. & Clin. Med. **24**: 495, 1939.

CUTANEOUS HYPERSENSITIVITY TO IODINE*

JOHN L. JACOBS, M.D., AND ABRAHAM COLMES, M.D.
BOSTON, MASS.

THE occurrence in certain individuals of a local or contact dermatitis following the application of iodine has been described by many authors. Some[1-4] speak of the symptoms as toxic in origin; others[5-12] consider them as manifestations of hypersensitiveness; and two authors[13] do not indicate their opinion. Except for one instance[7] of a patient giving a delayed reaction by scratch test to Lugol's solution diluted 1:16 (and not to 10 per cent potassium iodide), confirmatory skin tests have not come to our attention.

Most of the reports on iodine dermatitis are based upon single observations; this indicates the infrequent occurrence of this condition.

Recently a medical student, hypersensitive to iodine, became available for study. It seemed desirable, therefore, to investigate his abnormal reactivity in some detail.

CASE HISTORY

The subject for study, to be referred to hereafter as J. A. B., had no past history of allergy. Family history included a maternal aunt with asthma and a father suffering from migraine. Five years previous (at the age of 18) the patient first noticed dermatitis following application of tincture of iodine. Not long before that he had fallen on a cinder track, and his injury had been treated by a local application of tincture of iodine over a large area on his leg. Since then the application of iodine tincture has regularly caused a local dermatitis confined to the area exposed.

EXPERIMENTAL

A preliminary test confirmed the fact that tincture of iodine (7 per cent iodine, 5 per cent potassium iodide), which gives no reaction on normal controls, produced reddening in our patient within seven hours after the application of one drop to the skin. By the second day blisters had developed, with thickening of the epidermis. These blisters exudated serum for approximately two days and then began to dry up. The site of application could be detected for some time; after a month it was still slightly reddened and covered with scabby scales. A 1:10 dilution of the above tincture in alcohol also produced a similar reaction, though of lesser intensity.

Another series of tests was performed on this patient with a tenth normal iodine solution (1.3 Gm. iodine and 3.0 Gm. potassium iodide in 100 c.c.). Tenth normal iodine solution does not irritate the skin of normal individuals, but in

*From the Departments of Pathology and Bacteriology, and Medicine, Tufts College Medical School, Boston.

Received for publication, November 1, 1939.

This investigation was aided by a grant from the Charlton Research Fund of the Tufts College Medical School.

this patient it caused a reaction which was, however, less marked than that elicited by the iodine tincture. A few slightly raised, pale pink papules were present at twenty-four hours; they continued to increase in number and intensity of coloring so that after forty-eight hours the whole area affected was covered with pinkish elevations. A 1:10 dilution of tenth normal iodine solution failed to produce a reaction.

Powdered potassium iodide and a 1:1 solution of potassium iodide in distilled water failed to give a positive patch test. On the other hand, 0.5 per cent iodine salve in vaseline (crystals finely ground and mixed with vaseline in a mortar) which contained no potassium iodide, produced a marked delayed reaction. These results are tabulated in Table I.

TABLE I

SENSITIZATION TO IODINE

All recorded readings were made on the day following the test. In the patch tests an amount was used equivalent to the bulk of a pea; in the intracutaneous tests 0.02 c.c. was injected. Figures refer to millimeters.

TYPE OF TEST	SUBJECT	TEST PERFORMED WITH: 0.5% IODINE IN VASELINE	TINCTURE: 7% IODINE WITH 5% POTASSIUM IODIDE	5% IODINE IN ALCOHOL	SATURATED POTASSIUM IODIDE SOLUTION
Patch	J. A. B.	Pink, elevated	Pale pink, slightly elevated, spotty; 2 small vesicles	Negative	Negative
	Control 1	Negative	Negative	Negative	Negative
	Control 2	Negative	Negative	Negative	Negative

TYPE OF TEST	SUBJECT	TESTED WITH: IODINE, 1:1,000, IN SALINE	IODINE, 1:10,000, IN 0.9% POTASSIUM IODIDE	POTASSIUM IODIDE, 1:100	DIIODOTYROSINE 1:200
Intracutaneous	J. A. B.	4, pale pink slightly elevated	Negative	Negative	Negative
	Control 1	3, pale pink spot	Negative	Negative	Negative
	Control 2	3, faintly pink faintly elevated	Negative	Negative	Negative

When a vaseline salve containing only 0.25 per cent iodine was applied, no reaction occurred, whereas a 1.0 per cent salve, similarly prepared, caused irritation to normal skin. An attempt was made to increase this limited range of practicable ointment concentrations by the use of a method previously described for poison ivy hypersensitiveness in guinea pigs.[14] In this technique a high concentration of the test substance is applied, being removed after a short time by a suitable agent. To this end a 5.0 per cent ointment of iodine in vaseline, carefully ground and otherwise prepared as above, was applied to the forearm of our patient and washed off after twenty to thirty minutes with soap and water, followed by tenth normal sodium thiosulfate solution. The test was read the next day. Controls were uniformly negative, whereas J. A. B. showed a marked reaction of the type described above (Table III). With the above technique, ointment concentrations up to 10.0 per cent iodine may be used. In this way differences between the sensitized individual and normal controls were made more striking.

In contrast to the markedly positive patch tests to iodine salve, intracutaneous tests on J.A.B (0.02 c.c.) with a 1:1,000 solution of iodine were essentially negative. It should be pointed out, however, that this concentration of iodine is slightly less than the weakest solutions capable of giving a patch test in this individual. An intracutaneous test with 1.0 per cent potassium iodide was likewise negative.

In order to gain further information concerning the mechanism of these reactions, it seemed important to study the response of J. A. B. to iodinated protein, since a positive reaction would indicate that the iodine had combined with protein in the body to form an antigen containing the specific group of iodoproteins, diiodotyrosine. It is known that this reaction can easily take place under these conditions,[15] and reactions to formolized proteins in an individual hypersensitive to formaldehyde have been reported by Horsfall.[16] Accordingly, several preparations of iodinated human serum were made by Wormall's method,[17] and various tests were carried out on this patient and on suitable controls. A typical protocol is given in Table II.

TABLE II

TESTS WITH IODINATED PROTEIN

The readings are after fifteen minutes for the immediate reactions and after twenty-four hours for all others. Dilutions were made in saline and are expressed in terms of dry solids. Human serum was used in all the serum preparations. Measurements are in terms of millimeters.

SUBJECT	INTRACUTANEOUS TESTS WITH IODINATED SERUM				PATCH TESTS WITH:	
	0.05%		1.0%		PRECIPITATED IODINATED SERUM (WET SOLID)	DIIODOTYROSINE POWDER
	IMMEDIATE	DELAYED	IMMEDIATE	DELAYED		
J. A. B.	Wheal 8 Flare 19	8, pale pink, slightly elevated	Wheal 12 Flare 50	45, faintly pink, slightly thickened	Negative	Negative
Control 1	Wheal 9 Flare 20	3, pale pink spot	Wheal 11 Flare 50	30, faintly pink, faintly thickened	Negative	Negative
Control 2	Wheal 8 Flare 20	4, faintly pink	Wheal 7 Flare 40	35, very faintly pink, no thickening	Negative	Negative

It will be noted that, as far as immediate reactions are concerned, no marked differences were noted between J. A. B. and the controls. This is interesting because strongly positive reactions would be expected in human hypersensitivity to iodinated protein. Such differences in delayed reactions as occurred may not have been enough to be significant. While these tests were being carried out, it happened that another individual, J. B. H., hypersensitive to formaldehyde, was available. J. B. H. was tested for hypersensitivity to formolized human serum, but, as in the above instance with iodinated proteins, the outcome was essentially negative.

Failure of patch tests with precipitated iodinated protein, and diiodotyrosine, the specific grouping in iodinated proteins, was quite definite. Intracutaneous tests with diiodotyrosine were likewise negative (Table I). No generalized reaction was observed following any of the tests, although local reactions from

the patch tests at times were exceedingly severe. The responses observed were exclusively of the delayed type.

Since hypersensitiveness is a highly selective phenomenon, it was of interest to learn whether idiosyncrasy to iodine is subject to the same law of specificity. To obtain this information cross tests were performed with iodine and formaldehyde on J. A. B. and J. B. H. The method of testing and results noted are given in Table III.

TABLE III

SPECIFICITY OF IDIOSYNCRASY TO IODINE

Readings made on the day following the test

SUBJECT	TESTED WITH:		
	5% IODINE IN VASELINE WASHED OFF AFTER 15 MINUTES WITH SODIUM THIOSULFATE	LANOLIN SALVE CONTAINING 10% FORMALDEHYDE —NOT WASHED OFF	LANOLIN—NOT WASHED OFF
J. A. B.	Pale pink, slight vesiculation	Negative	Negative
J. B. H.	Negative	Pink, thickened, and rough	Negative
Control 1	Negative	Negative	Negative
Control 2	Negative	Negative	Negative

It will be observed that with these two substances, iodine and formaldehyde, the specificity was definite. J. A. B., hypersensitive to iodine, had a pink, slightly vesiculated lesion twenty-four hours after treatment with iodine salve, but gave no trace of reaction with formaldehyde salve. In J. B. H. the opposite reactions were observed. Thus these idiosyncrasies appear to be as specific as other immunologic phenomena. Since hypersensitivity to lanolin has been reported, a control test of this substance was included.

DISCUSSION

Interpretation of these studies is necessarily limited by the fact that they were obtained from a single experimental subject. However, certain points seem to be clear. The condition known as contact sensitivity to iodine is a manifestation of hypersensitiveness and is characterized by strict specificity. All intracutaneous tests with iodine solutions or iodinated protein were negative or equivocal.

In regard to the failure to obtain immediate reactions to intracutaneous injections of iodinated protein, it should be considered that a single negative result of this sort does not carry much weight. However, the tests were repeated a number of times with different preparations and concentrations, and the opportunity for technical errors would not seem to be great. It would seem that Wolff-Eisner's hypothesis[18] that these idiosyncrasies are due to the formation of an antigen by the combination of iodine with body constituents has not yet been proved. It may be recalled, however, that reactions with protein combinations have been obtained in guinea pigs[19] sensitized to simple chemical substances.

The absence, noted in Table I, of a dermal reaction to a simple alcoholic solution of iodine (no potassium iodide) was observed twice but needs further confirmation since tests were performed with a minimal quantity of material in an attempt to avoid unnecessary reactions in this very hypersensitive patient.

SUMMARY

An individual giving marked contact reactions with iodine was examined. The specificity of the reaction and methods of testing were studied. Intradermal tests were essentially negative. No conclusive evidence of sensitivity to potassium iodide, diiodotyrosine, or iodinated protein was observed.

REFERENCES

1. deBroe, M. Patris: Intoxication Iodique Mortelle, Arch. de méd. et pharm. mil. **57**: 96, 1911.
2. Herzog, W.: Zur Anwendung der Iodtinktur, München. med. Wchnschr. **61**: 2319, 1914; The Dangers of Tincture of Iodine as a First-Aid Dressing, Brit. M. J. **1**: 441, 1915.
3. Bryan, R. C.: One Hundred Prostatectomies, Virginia M. Monthly **48**: 338, 1921.
4. Jones, O. T.: Dermatitis Consequent on Application of Iodine, Brit. M. J. **1**: 14, 1931.
5. Rutherford, W. J.: Excessive Reaction Following the Use of Tincture of Iodine, Brit. M. J. **1**: 846, 1915.
6. Clifford, S. H.: Iodine Hypersensitization, Boston M. & S. J. **195**: 931, 1926.
7. Brack, W.: Untersuchungen an einem Fall von Iodüberempfindlichkeit, Dermat. Ztschr. **56**: 26, 1929.
8. Alexander, R. C.: Fatal Dermatitis Following Use of Iodine Spirit Solution, Brit. M. J. **2**: 100, 1930.
9. Brenas and Weis: Dermite Bulleuse Géante de l'abdomen chez un Enfant, Bull. Soc. franç. de dermat. et syph. **40**: 19, 1933.
10. Garrido, J. A.: Iodism Following Percutaneous Application of Iodine, El Siglo. Med. **97**: 338, 1936.
11. Seymour, W. B., Jr.: Poisoning From Cutaneous Application of Iodine, Arch. Int. Med. **59**: 952, 1937.
12. Rowell, H. G.: Surgical Importance of Iodine Idiosyncrasy and Poisoning, Surg., Gynec. & Obst. **36**: 219, 1923.
13. Biesalsky: Discussion of a Case, Deutsche Gesellsch. f. Chir. **39**: 34, 1910; Moszkowicz, Ibid., p. 40.
14. Jacobs, J. L., and Welcker, M. L.: A Method of Testing Guinea Pigs for Hypersensitiveness to Rhus toxicodendron, Proc. Soc. Exper. Biol. & Med. **38**: 726, 1938.
15. Jacobs, J.: Serological Studies on Iodinated Sera, I. Precipitins and Precipitinogens, J. Immunol. **23**: 361, 1932.
16. Horsfall, F. L., Jr.: Formaldehyde Hypersensitiveness, J. Immunol. **27**: 569, 1934.
17. Wormall, A.: Immunological Specificity of Chemically Altered Proteins, J. Exper. Med. **51**: 295, 1930.
18. Wolff-Eisner, A.: Ueber die Urticaria vom Standpunkt der neuen Erfahrungen über Körperfremdem Eiweissubstanzen, Dermat. Centralbl. **10**: 164, 1906-07.
19. Landsteiner, K., and Jacobs, J.: Studies on the Sensitization of Animals With Simple Chemical Compounds II., J. Exper. Med. **64**: 625, 1936.

ACUTE ENDOCARDITIS DUE TO AN ANAEROBIC PNEUMOCOCCUS*

ALVIN HOLLANDER, M.S., AND EVA LANDSBERG, M.D.
BROOKLYN, N. Y.

IN 1936 three strains of anaerobic pneumococci were described by Smith.[1] Of these, two different strains were isolated from the throat of a patient with chronic bronchitis and one was obtained by post-mortem culture from the lung in a patient with pneumonia. White[2] cites a personal communication from Avery regarding the occurrence of anaerobic pneumococci. We have not been able to find any other descriptions of anaerobic pneumococci. The following case is the first instance in which such organisms have been isolated from the blood stream during life and also the first case of acute endocarditis caused by an anaerobic pneumococcus.

REPORT OF CASE

D. L. (Hospital No. 221450), a 9-year-old boy, was admitted to the Pediatric Service on April 22, 1939, with the following history: Thirteen days prior to admission, he complained of a headache on the left side of his head. Daily fluctuations in temperature from 98.6° to 104° F. were observed. On the third day of his illness, there developed swelling and redness of the left eye, and pain in the neck without rigidity. Eight days before admission, a cardiac murmur was heard for the first time by the family physician. During the three days before admission four chills occurred, with temperature elevations reaching 105.2° F.

Physical Examination: The patient was well nourished, well developed, and acutely ill. There was injection of the nose and pharynx and slight enlargement of the cervical lymph nodes. The heart was not enlarged but there was present a high-pitched musical systolic murmur at the apex, transmitted to the axilla. The spleen was palpable 0.5 cm. below the costal margin and was firm but not tender. The liver was palpable 1.5 cm. below the costal margin. The swelling of the eye was no longer evident and the pains in the neck had disappeared. The laboratory data were as follows: hemoglobin 70 per cent, erythrocytes 3,570,000 per cmm., leucocytes 12,500 per cmm., with 82 per cent polymorphonuclear cells, of which 23 per cent were band forms, 14 per cent lymphocytes, and 4 per cent monocytes. The urine was negative.

Course: At 10:00 P.M. on the day of admission the patient had a chill with a rise of temperature to 105° F. A blood culture taken at this time showed a growth of anaerobic pneumococci after three days (the organisms will be described later). On the following day there developed a slight swelling and tenderness of the left knee and left shoulder and hip.

On April 24, two days after admission, the patient showed signs of meningitis with rigidity of the entire spine and neck. The Kernig sign could not be evaluated because of the pain in the left limb. Lumbar puncture revealed a turbid spinal fluid under slightly increased pressure. A cell count of 1020 per c.mm., with 93 per cent polymorphonuclear cells and 7 per cent lymphocytes was obtained. The sugar content was normal. A Gram stain of the fluid showed very rare gram-positive diplococci which failed to grow on culture. Blood culture revealed the same organism previously isolated.

Because of the presence of gram-positive cocci in the spinal fluid, intrathecal sulfanilamide and oral sulfapyridine therapy were instituted. Spinal fluid withdrawn immediately before the first intrathecal injection of sulfanilamide contained 760 cells per c.mm., 75 per cent being polymorphonuclear cells and 25 per cent lymphocytes. This specimen showed no organisms in the direct smear and no growth on culture.

*From the Division of Bacteriology, Departments of Laboratories and Pediatrics, the Jewish Hospital of Brooklyn.

Received for publication, November 3, 1939.

By the next morning the temperature had dropped to below 100° F. The tenderness in the lumbosacral region and left leg and the nuchal rigidity had almost completely disappeared. Roentgenographic examination of the hip and pelvis was negative. There was present definite haziness in the left frontal, ethmoidal, and maxillary sinuses. (Cultures were not obtained directly from the sinuses, but nose and throat cultures showed no anaerobic pneumococci.) During the next week the temperature remained below 100° F. and the child's condition appeared to improve.

On May 2 a tender erythematous area, 3 cm. in diameter, was seen on the dorsum of the right foot. On May 4 and May 6 the patient had chills with a positive and negative blood culture, respectively. On the latter date he complained of severe pain in the left hand which showed tenderness and swelling of the metacarpal region and redness and swelling of its volar surface. In the evening of the same day a purpuric rash appeared on the volar surfaces of both hands and the plantar surface of the right foot. The rash consisted of reddish-violet spots, about 0.5 cm. in diameter, which were not elevated and which faded on pressure. Cultures of these areas were negative. The spleen was palpable 3 cm. below the costal margin and was hard, firm, and not tender. The fundi were normal. By May 14 the rash was almost gone. The patient had a yellowish pallor but no jaundice. There was pain in the right elbow which, however, was not red or swollen. The murmur was much louder and scattered sibilant râles were heard over both lung fields. The liver could now be felt 4 cm. below the costal margin.

On May 16 the face was flushed and had a cyanotic tinge. There was present in each eye a hemorrhage at the margin between the inferior bulbar and palpebral conjunctivae. On May 20 the patient became irrational and delirious. Convulsive seizures occurred, starting on the left side and becoming generalized in a few seconds. During these seizures the right arm and leg were spastic. The course continued steadily downhill and the patient died on May 22. A lumbar puncture shortly before death showed a turbid fluid with innumerable gram-positive diplococci. On culture these organisms proved to be identical with those isolated from the blood. Consent for autopsy was not given.

BACTERIOLOGIC STUDIES

The organism was a small gram-positive lancet-shaped diplococcus which could be grown only under anaerobic conditions. The colonies, like those of pneumococci, were flat and surrounded by an area of greenish discoloration. It was bile soluble but did not give a "Quellung" reaction with the sera of the known types. Fermentation with acid and clot formation occurred in inulin, galactose, lactose, maltose, saccharose, mannite, sorbitol, dextrose, salicin, glycerol, and levulose serum media. The strain isolated by Smith differed in that no reaction took place in mannite, and acid only was formed in sorbitol and glycerol.

Per se the organism was not pathogenic for mice. When injected together with mucin, however, the mice were killed in eighteen hours by a suspension containing 150,000,000 organisms per cubic millimeter and positive cultures were obtained from the peritoneal exudate and heart's blood. In rabbits the intravenous injection of as much as 2 c.mm. of an eighteen-hour broth culture caused a transient bacteriemia which did not have a fatal outcome. When the animals were sacrificed six weeks after the injection, the only lesions present were a few small abscesses in the liver. Intracutaneous inoculation of 1 c.mm. produced the same picture except for the presence of a huge local abscess around the site of the injection. Guinea pigs were killed by the intraperitoneal injection of 1 c.mm. of an eighteen-hour broth culture, although 0.1 c.mm. was not fatal.

Mice infected with the organism-mucin mixture were not protected by either sulfapyridine or sulfanilamide. Control experiments with a virulent pneumococcus revealed that mice could not be protected against suspensions of the same density, although the therapy was effective against smaller numbers of lethal doses. Obviously, no conclusion can be drawn concerning the susceptibility of our strain to sulfapyridine.

Rabbits were immunized by repeated intravenous injections of a suspension of killed organisms. The antiserum produced gave positive "Quellung," agglutination, and protection tests with the homologous strain. No "Quellung" reaction was obtained with any of the 32 known serologic strains, and agglutination and protection tests with types I, II, V, VI, and XIV were negative.

For approximately four months after its isolation, the organisms could be grown only under anaerobic conditions. After this period, however, it began to grow aerobically. At first there was a scanty growth but within five days it grew luxuriantly. The aerobic strain was identical with the anaerobic in its fermentation and serologic reactions, its virulence for mice, and its behavior to sulfapyridine.

DISCUSSION

A case of acute endocarditis and meningitis caused by an anaerobic pneumococcus is reported. The onset with headache and swelling of the eye and the roentgenographic evidence of infection in the sinuses indicate that these were the primary foci, although in the absence of positive cultures this cannot be stated definitely.

Since soluble sulfapyridine was not then available, intrathecal sulfanilamide was given and maintained at a level of 7.6 mg. per cent for five days. The effect of this therapy is difficult to evaluate. As seen from Table I, the second spinal fluid was already sterile and the number of cells had fallen from 1,020 to 760 per cubic millimeter. The subsequent fluids, except for the last one, were all sterile and showed a steady decrease in the cell count. The intrathecal therapy was discontinued when the number of cells had fallen to 29.

Concurrently with the intrathecal sulfanilamide, oral sulfapyridine was instituted. On April 26, two days later, a blood level of 15 mg. per cent was reached. During the next week a total of 725 grains of sulfapyridine was given. The temperature in this period was below 100° F. On May 4 the patient suffered a chill with a rise in temperature to 104.2° F. A blood culture at this time was positive. The concentration of sulfapyridine in the blood which had been 9.4 mg. per cent the previous day had fallen to 5.7 mg. per cent. The spinal fluid showed no organisms and had only 21 cells per cubic millimeter. Since the temperature remained high and the blood sulfapyridine could not be raised above 6.9 mg. per cent by oral administration, a soluble sulfapyridine compound (7 per cent solution) was given intravenously. After two intravenous injections the temperature curve appeared to take a downward trend. On the next day, despite a sulfapyridine level of 11.1 mg. per cent, a positive blood culture was obtained. For the next two days the temperature ranged between 99° and 101° F. Following the third injection, however, a rise in temperature occurred and the intravenous therapy was dis-

TABLE I

SPINAL FLUIDS

DATE	PRESSURE	APPEARANCE	SMEAR	CULTURE	CELLS	SULFANILAMIDE CONCENTRATION (MG. %)
4/24	Slight increase	Turbid	Gram-positive diplococci (rare)	No growth	1,020 (93% polys.) (70% lymph.)	
4/25	Slight increase	Turbid	No organisms	Sterile	760 (75% polys.) (25% lymph.)	5.0
4/26	Slight increase	Turbid	No organisms	Sterile	860 (69% polys.) (31% lymph.)	6.5
4/27	Slight increase	Clear	No organisms	Sterile	220 (79% polys.) (21% lymph.)	7.9
4/28	Normal	Clear	No organisms	Sterile	96 (80% polys.) (20% lymph.)	8.4
4/29	Normal	Clear	No organisms	Sterile	70 (52% polys.) (48% lymph.)	7.8
4/30	Normal	Clear	No organisms	Sterile	29 (38% polys.) (62% lymph.)	8.4
5/ 1	Normal	Clear	No organisms	Sterile	32 (37% polys.) (63% lymph.)	
5/ 4	Slight increase	Clear	No organisms	Sterile	21 (48% polys.) (52% lymph.)	
5/22	Slight increase	Turbid	Innumerable gram-positive diplococci	Anaerobic pneumococcus		

TABLE II

BLOOD

DATE	CULTURE	SULFAPYRIDINE CONCENTRATION (MG. %)
4/22	Anaerobic pneumococcus	
4/24	Anaerobic pneumococcus	
4/26		15.0
5/ 3		9.4
5/ 4	Anaerobic pneumococcus	5.7
5/ 6	Sterile	
5/ 9	Sterile	6.9
5/11	Anaerobic pneumococcus	11.1
5/18	Anaerobic pneumococcus	3.2

continued. The patient continued to receive 60 grains of sulfapyridine daily by mouth. Nevertheless, one week later the blood level was only 3.2 mg. per cent. From Table II it is obvious that there was no relationship between the outcome of the cultures and the concentration of sulfapyridine in the blood. This fact was given added support by our experiment which showed the resistance of the organism to the drug.

Data concerning the frequency of the occurrence of anaerobic pneumococci is not available, since they are, as a rule, not sought for routinely. Our observations suggest that further investigation along these lines is needed.

SUMMARY

1. A case of acute endocarditis and meningitis caused by an anaerobic pneumococcus of a new serologic type is reported.

2. The identity of the organism was established by its solubility in bile, fermentation of inulin, and "Quellung" reaction with an homologous immune serum.

3. These organisms were not pathogenic for mice, which could be killed by the injection of a heavy suspension in mucin.

4. Mice infected with mucin suspensions of these organisms were not protected by either sulfanilamide or sulfapyridine.

We wish to express our gratitude to Mrs. Anita Rosenfeld for her technical assistance.

ADDENDUM: In a recent article Walter and her co-workers[3] described a new type of pneumococcus which they designated as type 33. Miss Walter was kind enough to examine our strain and found it to be of the same type.

REFERENCES

1. Smith, F.: Anaërobic Pneumococcus, Brit. J. Exper. Path. 17: 329, 1936.
2. White, B.: The Biology of Pneumococcus, New York, 1936, The Commonwealth Fund, p. 44.
3. Walter, A. W., Blount, K. D., Beattie, M. W., and Cotler, H. Y.: J. Infect. Dis. 66: 181, 1940.

A QUALITATIVE AND QUANTITATIVE STUDY OF ATMOSPHERIC POLLEN AT MOSCOW, IDAHO

JEAN B. DAUBENMIRE, M.S., MOSCOW, IDAHO

THE collections of atmospheric pollen grains used in this study were obtained by exposing gelatin-coated microscope slides on top of Morrill Hall on the campus of the University of Idaho at Moscow. The campus is on a hill, and the roof of Morrill Hall, a four-story building, is high enough that the local vegetation has a minimum effect upon the composition of the pollen of the atmosphere.

An attempt was made to collect pollen for every twenty-four-hour period. This was not always possible, and as many as six days elapsed between some of the samples.

The slides were prepared according to Wodehouse's[1] glycerin jelly method, and the stain, basic fuchsin, was prepared according to the method of Chamberlain.[2] The slides were exposed 6 inches below the flat roof of a small especially constructed shelter. After exposure the slides were stored in cream sample bottles to keep them from gathering more pollen and dust before the cover slips were applied. They could be stored in this way indefinitely if rainy weather did not excessively moisten the gelatin so as to permit mold spores to germinate.

Wodehouse's[1] key for the identification of pollen grains was used, and as further aids in identification, observations were made on the phenology of plants in the Moscow vicinity, and reference mounts were prepared from fresh or from herbarium specimens.

Slides were exposed from February 19 until December 2, although no pollen was found on them before March 5 or after November 30.

Received for publication, November 6, 1939.

The first pollen caught was three grains of grass pollen which appeared on March 5 (see Chart 1). Since no other grains of this type were caught until late in April, these grains were apparently produced the previous year. Other pollens appearing too early for the regular seasons were from Pinus, Artemisia, Chenopodiaceae, or Amaranthaceae, and of the Anthemis and Rumex types. Pine sheds its pollen most abundantly in June, but old staminate cones were found shedding pollen in midwinter of 1939 shortly after this study was discontinued. This indicates a possible source of at least some of the stray grains collected out of the normal season. Duke and Durham[3] state that rain washes from the pollen grains the active principle causing allergy, so that these few out-of-season grains can be completely ignored in using the data in the treatment of pollinosis.

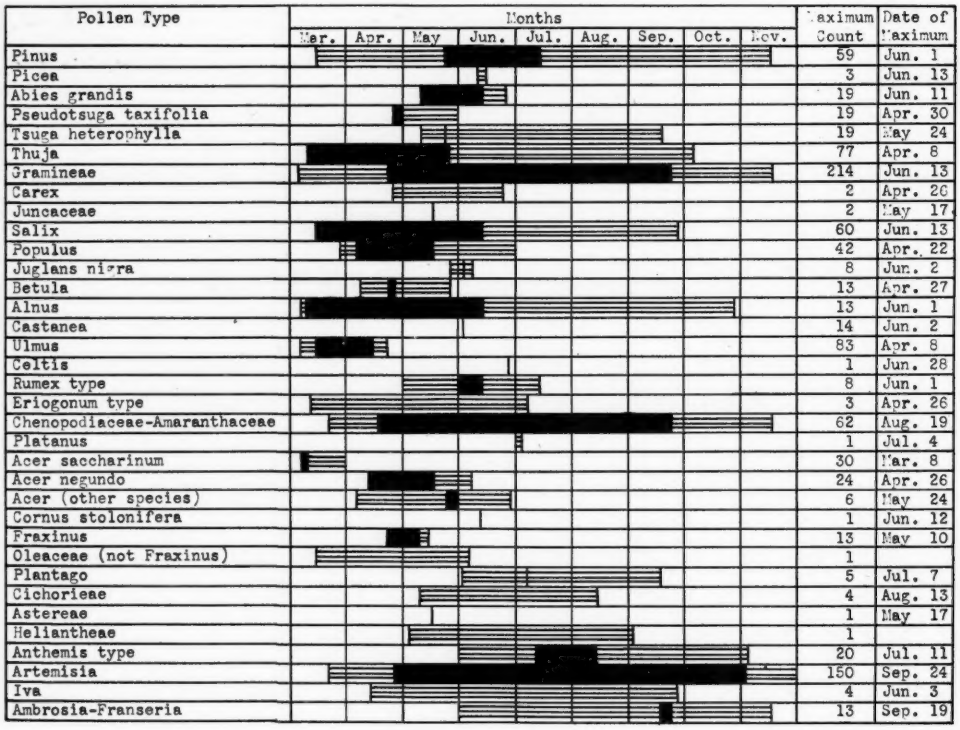


Chart 1.—Pollen types collected at Moscow during 1938. Shaded area indicates the total span of the pollination season; black area indicates the interval between the first and last days on which five or more grains fell on the sample area of gelatin (3.24 cm.).

Although pollen analysts in other regions have distinguished between species belonging to the Chenopodiaceae and Amaranthaceae, this distinction was found impossible at Moscow. Table I, showing the measurements of a series of grains collected in early summer, indicates the difficulty encountered.

The local flora includes about twenty species in the Chenopodiaceae (divided among Atriplex, Chenopodium, and Salsola) and four species of Amaranthus. The data in Table I show that the measurements of the pollen grains of this particular flora overlap to an extent that the distinction of the species is practically impossible.

TABLE I

NO. OF GRAINS	DIAMETER OF GRAIN	DIAMETER OF PORE	DISTANCE BETWEEN PORES
	mμ	mμ	mμ
1	21.58	1.66	4.15
1	23.24	2.49	5.81
1	23.24	3.32	5.81
1	24.90	1.66	2.49
1	24.90	1.66	4.15
1	24.90	2.49	9.96
1	24.90	2.49	3.32
1	24.90	2.49	4.49
2	24.90	2.49	4.15
1	24.90	2.49	6.64
2	24.90	3.32	6.64
1	24.90	3.32	4.98
1	24.90	3.32	8.30
1	26.56	2.49	6.64
1	26.56	3.32	6.64
2	26.56	3.32	4.98
1	26.56	4.15	5.81
1	28.22	3.32	4.98
1	28.22	3.32	8.30
1	29.88	1.66	2.49
1	29.88	3.32	4.98
1	29.88	2.49	4.98

Four per cent of the total grains collected during the year were received in a crushed condition, or were of unrecognized types.

Examination of weather records,[4] particularly the deviations from normal precipitation and temperature, shows that during the months when this study was made, the weather was slightly drier and warmer than the forty-six-year averages. According to Durham,[5] the dates of onset, crises, and close of the pollen seasons are practically the same at a given locality each year, except in cases of extreme drought. The weather of 1938 so closely approximated the normal climate that the pollen data presented in Chart 1 are probably typical of average years.

I wish to thank Dr. R. F. Daubenmire for suggesting the problem and directing the research, and Dr. A. E. Braun and Mr. Arthur Nelson for assistance in exposing slides during my absence from Moscow.

REFERENCES

1. Wodehouse, R. P.: Pollen Grains: Their Structure, Identification and Significance in Science and Medicine, New York, 1935, McGraw-Hill Book Co., p. 574.
2. Chamberlain, C. J.: Methods in Plant Histology, ed. 5, Chicago, 1932, Univ. of Chicago Press, p. 405.
3. Duke, W. W., and Durham, O. C.: Pollen Content of the Air, J. A. M. A. **90**: 1529, 1928.
4. United States Department of Agriculture Weather Bureau: Climatological Data, Idaho Section, Annual Summary **41** (13): 57-62, 1938.
5. Durham, O. C.: The Pollen Content of the Air in North America, J. Allergy **6**: 128, 1935.

INTRAMUSCULAR ADMINISTRATION OF SODIUM SULFAPYRIDINE*

LYNN T. HALL, M.D., EDWARD THOMPSON, M.D., AND
RAYMOND J. WYRENS, M.D., OMAHA, NEB.

INTRAVENOUS and oral administrations of sodium sulfapyridine have been up to now the only routes used in the United States. Most American clinicians have administered the drug intravenously,[1] and many reports have appeared in the literature on this means of administration.[2, 3, 5, 6] However, the intramuscular route offers certain advantages over the intravenous route if the drug can be given with equal safety. Gaisford[4] and others have preferred to give the drug intramuscularly.

Before recommending this type of treatment, one should consider certain experimental work. Recent reports of experimental intramuscular injections in guinea pigs showed such therapy to be dangerous and impractical, at least in the guinea pig. Necrotic sloughs occurred frequently, and local reactions were many.[5] In human muscles, however, the mass of muscle is larger and the total quantity of extracellular space that apparently contains large quantities of chloride is greater, thus providing a factor of safety. Those clinicians opposed to the intramuscular route of administration also contend that this method is likely to produce local irritation and that, even if the drug is deposited into the muscle, a depot may be formed from which prolonged absorption can take place. Furthermore, because of the high pH (11.5) of the material used as compared with normal body fluids, it is argued that this solution is far too alkaline and caustic to be employed with safety. We have not found this to be so.

During the past five months 25 patients have been treated with more than 350 intramuscular injections of the drug.

METHOD OF ADMINISTRATION

A 33⅓ per cent solution of sodium sulfapyridine in sterile water was prepared freshly with each dose of the drug. This preparation was injected deeply into the gluteal or thigh muscles, care being taken that none of the material was deposited into the subcutaneous tissue through the needle tract either upon injection or upon withdrawal of the needle. The schedule of dosage followed closely that used in the oral administration of the drug. Each cubic centimeter of the solution represents 0.33 Gm. At the first injection from 6 to 10 c.c. were given, followed by 3 c.c. every four hours, until the temperature was normal for twenty-four hours. Thereafter the dose was reduced one-half, and its administration was continued for the next five or six days.

*From the Department of Medicine, College of Medicine, University of Nebraska.
The material used in this study was furnished by Merck & Co.
Received for publication, June 27, 1940.

DISEASES STUDIED

Treatment was not limited to cases of pneumococcal infections but included several other diseases, with varying degrees of clinical success. They are included in this series to demonstrate the efficacy of the route of administration rather than to stress clinical effects of the drug.

ANALYSIS OF DATA

Table I shows a record of treatment of 25 persons with infectious diseases, 14 of whom had pneumonia. Diagnoses were confirmed by culture, typing, and x-ray studies whenever possible. Concentration studies of the drug in the blood were likewise made, and these are recorded. The blood levels recorded in the table represent figures obtained after or during intramuscular injections and before oral administration had been started. These blood levels closely parallel those obtained with the same amount of the drug administered orally. It will be noted that the temperature responses are almost identical with those following the oral administration of the drug. The clinical results appeared to be as good as those obtained by other methods of administration. Toxic effects were strikingly few or extremely mild when noted. In the 25 patients studied there were no local effects of irritation or tumefaction. The patients reported no more pain attendant upon the intramuscular procedure than has been noted after any other parenteral therapy in common use. The only toxic effects noted were four instances of nausea and vomiting; three of these patients had been vomiting before the drug was given. No instances of agranulocytosis, massive red blood cell destruction, or skin reactions were noted in the series.

DISCUSSION

It is not the purpose of this communication to urge the intramuscular administration of sodium sulfapyridine to the exclusion of other routes. However, if it can be shown that this method is a safe procedure, nearly everyone will acknowledge that the technical advantages of this route of administration are many.

Some investigators have noted that muscles are capable of storing metallic drugs as well as other materials with no apparent damage and that absorption takes place thereafter. If such theoretical depots or storage reservoirs are produced, they do not appear to cause demonstrable effects. It is also of interest that adequate levels (3 to 10 mg. per cent) of the drug in the blood may be obtained promptly by this method. Moreover—an important point—once the level is reached, it can be maintained for much more protracted periods than is the case with intravenous therapy. Fenn and his associates[7] in 1934 and Sandow[6] in December, 1939, pointed out that 15 per cent of muscle volume is extracellular space, in which all the chloride is believed to reside. If this is the case, it may furnish an explanation for the ease with which a muscle is able to handle the very alkaline sodium sulfapyridine solution.

The technical advantages of giving any drug intramuscularly are many if the procedure is otherwise safe. Gastrointestinal reactions to the drug are avoided. Nausea is usually a very constant symptom when the drug is given

TABLE I

SUMMARY OF DATA CONCERNING INTRAMUSCULAR USE OF SODIUM SULFAPYRIDINE

NO.	PATIENT	DIAGNOSIS	BACTERIOLOGY	NO. OF INJECTIONS	TOTAL AMOUNT OF DRUG (GM.)	BLOOD LEVEL (MG. %)	TEMPERATURE RESPONSE (F.°)	TOXIC EFFECTS	OUTCOME
1	H. B.	Lobar pneumonia	Positive blood culture Type III	32 in 11 days, none orally	Im.—34	3 to 5	104° to normal in 48 hr.	None	Well
2	A. B.	Lobar pneumonia	Type III	26 in 6 days, none orally	Im.—38	2.3 to 5.4	103° to 100° in 48 hr.	None	Well
3	A. M.	Lobar pneumonia	Type III	24 in 7 days, none orally	Im.—27	3.3 to 5.4	102° to normal in 48 hr.	None	Well
4	H. B.	Lobar pneumonia, pregnancy	Type I	41 in 12 days, none orally	Im.—44	4.4 (10 hours)	104° to normal in 54 hr.	None	Improved
5	J. M.	Lobar pneumonia	Type I	11 in 3 days, then orally	Im.—11 O—4		103.8° to normal in 24 hr.	Vomiting began before drug was given	Well
6	E. B.	Lobar pneumonia	Type II	1 dose intramuscularly, then orally	Im.—2.3 O—14		102° to normal in 3rd day	None	Well
7	R. E.	Lobar pneumonia	Type I	2 doses intramuscularly, then orally	Im.—4 O—19		103° to normal in 36 hr.	None	Well
8	J. K.	Lobar pneumonia	Type III	1 dose intramuscularly, then orally	Im.—2.3 O—41		104° to normal in 24 hr.	None	Well
9	W. C. B.	Lobar pneumonia	Type I	2 doses intramuscularly, then orally	Im.—4 O—23		104° to normal in 54 hr.	None	Well
10	W. R. B.	Broncho-pneumonia	Type VI	1 dose intramuscularly, then orally	Im.—2.3 O—12		104° to normal in 18 hr.	None	Well
11	S. V.	Broncho-pneumonia	Type II	1 dose intramuscularly, then orally	Im.—2.3 O—12		102° to normal in 30 hr.	None	Well
12	I. L.	Acute appendicitis, postoperative *pneumonia*	Type XVII	1 dose intramuscularly, then orally	Im.—2.3 O—8		102° to normal in 12 hr.	None	Well
13	T. L.	Fractured rib, broncho-pneumonia	Diplococci not typed	1 dose intramuscularly, then orally	Im.—2.3 O—29		101° to normal in 3 days	None	Well

14	W. S.	Pneumonia	Streptococcus	1 dose intramuscularly, then orally	Im.—2.3 O—11		102° to normal in 24 hr.	None	Well
15	L. C.	Peritonitis (pneumococcal?)		23 in 8 days, then orally	Im.—19.7 O—16	As high as 21.7	104° to normal in 6 days	None	Well
16	W. H.	Undulant fever	Positive agglutination	25 in 5 days, none orally	Im.—27	3.5 in 10 hr.	No definite response	Vomiting began before drug administration was begun	Still in hospital
17	H. F.	Hemiplegia, bronchopneumonia		36 in 10 days, none orally	Im.—46	2.2 to 2.7	None	None	Died
18	M. R.	Coronary thrombosis, pulmonary infarction		9 in 36 hr., none orally	Im.—10		Questionable	None	Improved
19	F. S.	Appendicitis with perforation		17 in 6 days, then orally	Im.—17 O—27	Less than 3	103° to normal in 3 days	None	Improved
20	E. P.	Appendicitis with peritonitis		5 in 2 days, none orally	Im.—6	6.4 after 24 hr.	None	None	Died
21	R. K.	Empyema; history of pneumonia preceding	No growth	25 in 10 days, then orally	Im.—29 O—4	4.8	None	None	Died
22	E. L.	Pansinusitis, glomerulonephritis		9 in 48 hr., none orally	Im.—9		102° to normal in 12 hr.	Moderate nausea and vomiting	Improved
23	B. T.	Addison's disease, pharyngitis		26 in 8 days, none orally	Im.—27	8.3	103° to 99°-100° in 24 hr.	Little	Improved
24	P. B.	Diabetes, abscess of leg	Nonhemolytic streptococcus	21 in 7 days, none orally	Im.—21	5.9	None	None	Died
25	D. S.	Typhoid fever	Positive Widal	23 in 8 days, none orally	Im.—25		Questionable	None	Well

by mouth, and the condition even progresses to vomiting, which precludes the administration of the drug. In the treatment of comatose patients it is obviously unwise to attempt to make them swallow drugs or to introduce the drugs into the stomach by tube, since in either instance the aspiration of the drug is likely. Old persons with tortuous and sclerotic vessels, infants, children, and women with inconspicuous or no ample sized veins are usually poor subjects for intravenous therapy. Likewise a safe alternative route of administration is always desirable in those cases in which any factor has made the oral or intravenous routes impracticable or impossible.

CLINICAL RECORD OF A TYPICAL CASE

Mrs. A. M., aged 65 years, was admitted to the University Hospital on Dec. 8, 1939, complaining of chills, fever, and cough with bloody sputum. Seventy-two hours before admission she had been suddenly seized with a chill which was soon followed by fever. She began to cough and shortly thereafter felt a sharp pain in the right chest which was aggravated by deep breathing and by coughing.

Her past history is irrelevant except for two attacks of lobar pneumonia, one in 1931, the other in 1934. Each time she made an uncomplicated recovery.

On admission her temperature was 101° F. and rose to 102° F. within two hours. There was limitation of motion of the right chest, with dullness and decreased breath sounds over its lower half. A few moist râles were heard over the same area. Leucocyte count was 15,200, of which 80 per cent were polymorphonuclear in type. Urine was negative. A roentgenogram of the chest, taken by the portable unit, revealed a diffuse density involving the middle third of the right lung field. Sputum examination revealed type III pneumococci.

At eleven o'clock on the night of admission 6 c.c. of 33⅓ per cent solution of sodium sulfapyridine were administered intramuscularly, followed in four hours by another 6 c.c. The following morning administration of 3 c.c. of solution every six hours was begun and continued for six days. On the morning after admission, after a total of 4 gm. of the drug had been received intramuscularly, examination showed a blood concentration of 3.3 mg. per 100 c.c. Two days later the blood level was 5.4 mg. per 100 c.c. and still later 4.1 mg. per cent.

The patient's temperature on the second day of admission ranged around 100.5° F.; the following morning it dropped to normal where it remained throughout the remainder of her hospital stay. The chest findings rapidly resolved and disappeared entirely by the seventh day after admission. After the second day the patient felt much better subjectively, and at no time was there any indication of local irritation at the site of injection. The patient was dismissed in good health on Dec. 17, 1939, her tenth hospital day.

CONCLUSIONS

1. Twenty-five cases have been analyzed, in which more than 350 intramuscular injections of 33⅓ per cent solution of sodium sulfapyridine were given.

2. Clinical results of the drug as manifested by blood levels and temperature response were parallel to those obtained by orally administered sulfapyridine.

3. Extreme care must be exercised to insure the deposition of all the drug into the muscle.

4. Toxic effects were minimal. Local evidences of irritation were not seen.

5. There are many technical advantages to this route of administration.

REFERENCES

1. Marshall, E. K., and Long, P. H.: The Intravenous Use of Sodium Sulfapyridine, J. A. M. A. 112: 1672, 1939.
2. Finland, M., Lowell, F. C., Spring, W. C., Jr., and Taylor, F. H. L.: Parenteral Sulfapyridine; The Intravenous Use of Sodium Sulfapyridine and a Report of Clinical and Laboratory Observations on the Use of Glucose-Sulfapyridine Solution, Ann. Int. Med. 13: 1105, 1940.
3. Abernethy, T. J., Dowling, H. F., and Hartman, C. R.: The Treatment of Lobar Pneumonia With Sulfapyridine and Sodium Sulfapyridine With Observations Upon Effective Blood Levels, Ann. Int. Med. 13: 1121, 1940.
4. Gaisford, W. F., Evans, G. M., and Whitelaw, W.: Parenteral Therapy With M. and B. 693, Lancet 2: 69, 1939.
5. Wilson, G. S., and Maier, Irene: Treatment With Sulphapyridine (M. and B. 693) of Guinea Pigs Infected With Brucella abortus, Brit. M. J., Jan. 13, pp. 47-50, 1940.
6. Sandow, A.: A Geometrical Determination of the Extracellular Space in Muscle, Proc. Soc. Exper. Biol. & Med. 42: 772, 773, 1939.
7. Fenn, W. O., Cobb, D. M., and Marsh, B. S.: Sodium and Chloride in Frog Muscle, Am. J. Physiol. 110: 261, 1934.

GIANT CELL TUMOR OF THE FRONTAL BONE

J. Jay Keegan, M.D., and Charles P. Baker, M.D., Omaha, Neb.

THE case of giant cell tumor of the frontal bone in a boy 13 years old is presented here because of the rarity of the lesion in bones which are of the membranous type. Review of the literature discloses only one previous case which was reported by Fraser[1] in 1931. This patient, aged 42 years, following trauma over the left forehead region, developed a mass. The mass was removed surgically and was found to be a soft tissue tumor which contained giant cells on section. Geschickter and Copeland[2] reviewed a series of 22 cases of giant cell tumor of the skull bones found at Johns Hopkins Hospital and found no instance of tumor which arose in membranous bone. All these cases could be related to centers of ossification arising in cartilage. They reached the conclusion that benign giant cell tumor could not occur in bones which arise membranously. Cotton[3] likewise observed that giant cell tumors do not occur in the bones of the skull.

CASE REPORT

A white boy, aged 13 years, was first seen on June 21, 1937, with a protruding mass in the right forehead region. This mass was first noted in 1935, at which time it was thought to be a swelling from an unrecognized trauma. However, progressive enlargement of the tumor mass, which was firm in consistency, painless, and not tender on palpation, discredited this impression. The mass gradually encroached on the right eye, displaced the eye, and restricted its movements. No history of trauma in the region of the tumor mass could be elicited.

The boy was healthy except for the tumor mass above the right eye. In this region there was a smooth, firm, right frontal tumor which measured 5 cm. in diameter and was elevated 1 cm. above the level of the surrounding external table of the frontal bone. The tumor was situated directly above the superior orbital ridge and external angular process of the frontal bone. Laterally it extended across the temporal ridge into the temporal fossa. The right eye was slightly exophthalmic and was displaced medially and downward, with restricted movements laterally and upward.

Received for publication, November 6, 1939.

Roentgenograms (Figs. 1 and 2) of the skull showed an extensive area of decalcification at the site of the tumor between the thinned inner and outer tables of the frontal bone. Medially a very thin, rounded plate of bone was thought to separate the tumor from a small frontal sinus. The frontal sinuses were clear and symmetrical except for slightly less lateral extension on the right. Laterally the tumor extended almost to the limits of the frontal

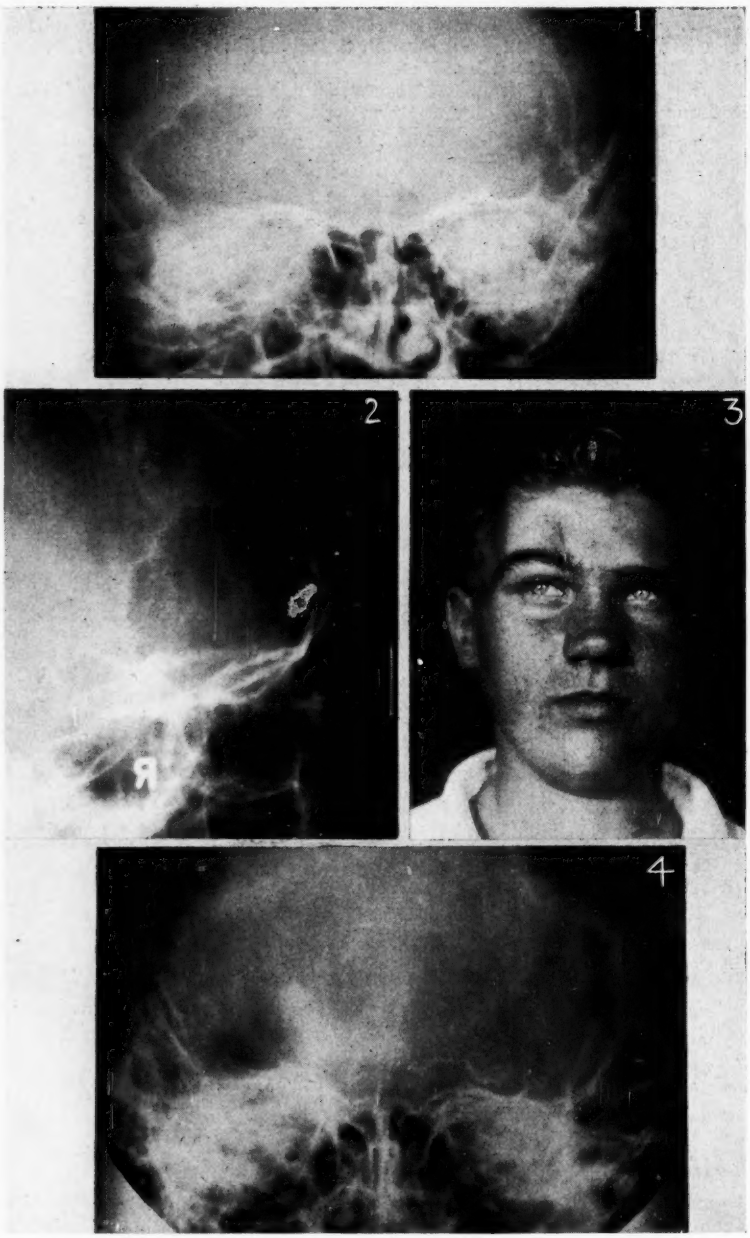


Fig. 1.—Frontal roentgenogram of skull showing encroachment of tumor on right frontal sinus and superior orbital plate.

Fig. 2.—Right lateral roentgenogram of skull showing extension of tumor into temporal fossa.

Fig. 3.—Photograph two years postoperative showing site of defect and normal right eye.

Fig. 4.—Roentgenogram two years and four months postoperative showing filling of bone defect.

bone at the articulation with the sphenoid and malar bones. The inner surface of the tumor was indefinitely outlined by a rounded, partly absorbed, inner table which seemed to protrude into the cranial cavity about 4 cm. The superior orbital plate was well defined but was depressed about 5 mm. from above and laterally. Some evidence of trabeculation could be seen within the tumor although its general density was little greater than the normal frontal sinus. A preoperative diagnosis of right frontal mucocele was made.

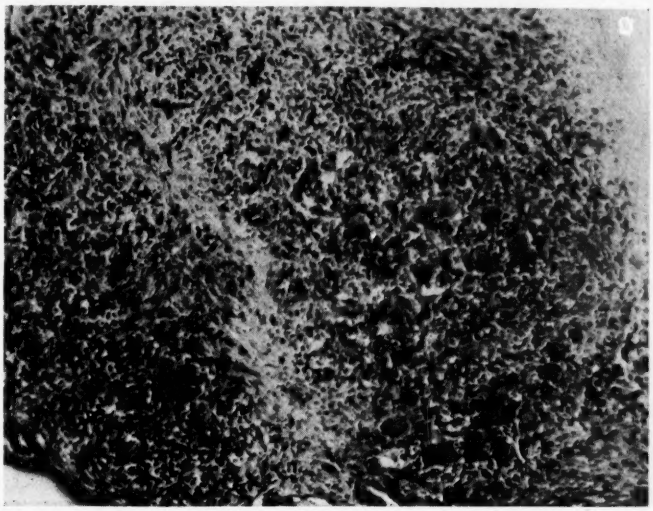


Fig. 5.—Low-power microphotograph of tumor showing numerous giant cells and fibrous stroma.

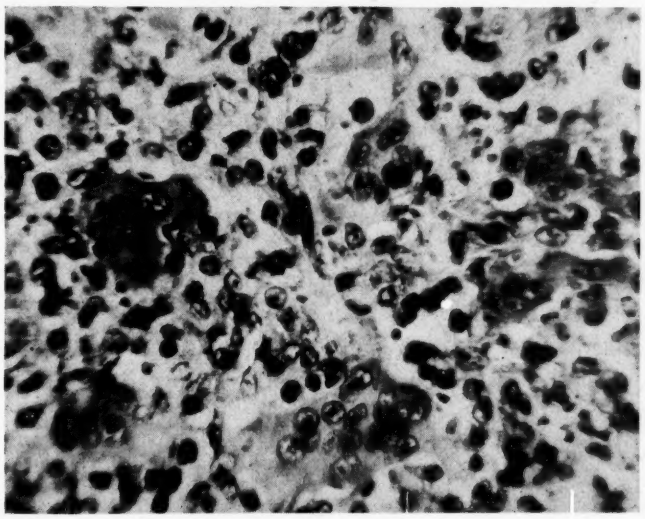


Fig. 6.—High-power microphotograph of tumor showing distribution of nuclei in giant cells.

At operation on July 19, 1937 an opening, 2 by 3 cm., was made through a somewhat roughened and almost perforated thin external table over the tumor. Moderately firm, grayish red tumor tissue was found filling the bone defect. An immediate pathologic report was giant cell tumor, possibly xanthoma. The tumor tissue was removed by blunt dissection and curettage with considerable bleeding. The inner table, which was found to be thin and eroded in places, was partly removed from the dura to reduce its impression on the frontal lobe of the brain and to permit some expansion of the frontal lobe into the large cavity where the tumor had been. The superior orbital plate was not removed where it had been depressed by

the tumor. Hemostasis was obtained with difficulty in the region of the lateral tumor bed by means of electrocoagulation. In this region and at other places in the wall some tumor tissue probably remained. The tumor cavity was filled with normal saline, and the scalp was closed without drainage.

Recovery occurred without aspiration or infection. Roentgen radiation therapy was given over the tumor area from July 26 to July 31, 1937, in a dosage of 1035 r. divided into six daily doses. The blood cholesterol determination on July 26, 1937, was found to be 150 mg. per cent, and the blood calcium was 8.8 mg. per cent, both within normal limits. Fluoroscopic examination of all the bones of the body disclosed no evidence of further bone lesions.

The boy has remained well for more than two years since the operation, and the area operated upon shows very little remaining deformity. The right eye does not appear exophthalmic, and its movements are normal (Fig. 3). Roentgenograms taken on Oct. 27, 1939 (Fig. 4) show the area of bone removed by operation surrounded by a smooth wall, considerably reduced in size with no evidence of recurrence of the tumor.

The tissue removed by the surgeon consisted of 32 Gm. of grayish red hemorrhagic tumor. The tissue was very friable and crumbled easily, except that removed from the periphery which was more firm and had more the appearance of fibrous tissue. These firmer areas extended into the central portion of the tumor.

On section the tumor was found to contain great numbers of multinucleated giant cells. In some areas there were from 20 to 40 giant cells in a low-power field (Fig. 5). The giant cells contained from 5 to 25 oval uniformly staining nuclei which had a tendency to be grouped in the center of the cells (Fig. 6). The cytoplasm of the giant cells was homogeneous and stained well with eosin. The giant cells were more abundant at the periphery of the tumor but were present to a lesser extent throughout the tumor. The stroma of the tumor was very cellular and consisted of round and spindle-shaped cells, the former being most numerous. The nuclei of the round cells were similar in appearance to the nuclei of the giant cells. The cytoplasm of the round cells was small in amount. The tumor contained many blood cells both inside and outside of blood vessels.

CONCLUSION

A case of benign giant cell tumor of the frontal bone has been presented. The location of this tumor in membranous bone is unusual since there is only one other case reported in the literature. The theory of Geschickter and Copeland for the origin of giant cell tumor from resorptive processes in cartilaginous bone seems quite reasonable but does not explain the origin of this tumor. We have been unable to find any evidence in the literature of embryology that cartilage is ever present in the frontal bone. We have made no study of the origin of giant cell tumor and have no theory as to the origin of this tumor.

REFERENCES

1. Fraser, J.: Benign Giant Cell Tumor of Bone, Clin. J. **60**: 20, 1931.
2. Geschickter, C. F., and Copeland, M. M.: Tumors of Bone, ed. 2, New York, 1936, Am. J. Cancer, chap. 12, p. 309.
3. Cotton, F. J.: Notes on Giant Cell Tumor of Bone and Cysts, New England J. Med. **210**: 1095, 1934.

CLINICAL CHEMISTRY

A THEORY OF PORPHYRINOGENESIS*

W. J. TURNER, B.S., M.D., NORTH LITTLE ROCK, ARK.

THERE is no one today who can say how hemoglobin is formed or from what sources its pigmented fraction, protoporphyrin, arises. After a century of study of hemoglobin metabolism there is still nothing known of its anabolic aspects. Between the absorption of food and the appearance of porphyrins and porphyrin compounds in the cells of the body and in excreta there lies an unexplored and undoubtedly important gap.

In the absence of any definite information as to how the various porphyrins arise, a number of hypotheses have been broached, all of which fail to satisfy the requirements of heuristic concepts in two major essentials. In the first place, they leave unexplained one or more of the major factors in the problem; in the second place, they offer no points for direct experimental attacks. Any theory of porphyrinogenesis must explain the following facts:

I. The occurrence of the following porphyrins: protoporphyrin 2, protoporphyrin 9, coproporphyrin I, coproporphyrin III, uroporphyrin I, and uroporphyrin III (Fig. I).

II. The normally great predominance of protoporphyrin 9.

III. The normal appearance of small and equal amounts of the two coproporphyrin isomers.

IV. The extremely rare occurrence of the uroporphyrin isomers.

As a matter of historical interest it is worthy of note that Günther,[1] in his 1911 monograph on porphyria, discussed the possibility that there might be a faulty catabolism of hemoglobin, with the production of "hematoporphyrin" in the liver. This view was shortly proved untenable, at least in its original form, by Fischer's[2] demonstration that the porphyrins excreted in porphyria are coproporphyrin and uroporphyrin, and that in any event they are of an entirely different isomeric type than is the blood porphyrin. Further studies on porphyria have also revealed that the enlarged liver of true congenital porphyria is not functionally impaired either as an excretory or as a metabolic organ,[3] and that acute porphyria not infrequently occurs without evidence of liver damage.[4, 5] It should not now require proof that the "urobilinogen" of the acute porphyric urine is not urobilinogen at all.[4, 6]

Following his demonstration that the feces of Petry contained the tetracarboxylic acid coproporphyrin, whereas the urine contained predominantly the octacarboxylic acid uroporphyrin, Fischer[2c] suggested that the former might arise by carboxylation of the latter in the kidney. This has been aban-

*From the Veterans Administration Facility, North Little Rock.
Published with permission of the medical director, Veterans' Administration, who assumes no responsibility for the opinions expressed.
Received for publication, October 26, 1939.

doned in view of the improbability that such a reaction could occur biologically. Further, it was soon found that uroporphyrin existed in Petry bone and in his ascitic fluid.[7]

By 1929, when Borst and Königsdorfer[8] published their monumental treatise on the pathologic examination of Petry, the München school was in agreement that the synthesis of the three main types of porphyrins ran independent courses. In particular, porphyria was regarded as an error of porphyrin anabolism.

Repeatedly suggested, even today, is the possibility that protoporphyrin may give rise to coproporphyrin. This is based on the old idea that the porphyrin excreted in porphyria is hematoporphyrin. It is to be said here that hematoporphyrin is an entirely artificial product of the chemical laboratory and does not occur in nature. The belief was a part of the theory that there might be a faulty breakdown of hemoglobin in the liver. In 1932 van den Bergh, Grotepass, and Revers[9] claimed that on perfusion of liver with a solution containing protoporphyrin the bile excreted contained coproporphyrin. This experiment has been widely quoted or tacitly used to support later proponents of a catabolic theory of coproporphyrin formation. Aside from the fact that it has never been repeated, and that Fischer has been unable to corroborate it,[10] the evidence presented by the authors is of the slightest sort. Fischer and Orth[10] say: "Möglicherweise händelt es sich hierbei aber um die leichte verständliche Bildung von Hämatoporphyrin IX, an Stelle des Koproporphyrin III." The objection must also be made that the catabolic theory entirely begs the question as to the origin of protoporphyrin.

Later Schreus[11] and Carrié[12] proposed a classification of diseases of porphyrin metabolism based on the type of porphyrin excreted. If type I porphyrins were formed, it would be considered a "primary" fault; if type III, it would be "secondary" to a faulty hemoglobin destruction. While some evidence has been presented for this view, and Vannotti,[13] in particular, leans to it, recent studies on porphyria have shown such a classification to be wholly untenable. Among the objections to it may be mentioned only the necessity of explaining how protoporphyrin may give rise to coproporphyrin; the failure to explain uroporphyrin or protoporphyrin formation; the failure to explain Dobriner's[14] evidence that coproporphyrin formation is associated with erythropoiesis rather than hemolysis; and the inability to explain the simultaneous excretion of both isomeric types of porphyrins, which is the rule rather than the exception.

The faulty hypotheses of the past have been inadequate in large part because of the lack of sufficient data on which to build soundly. Since the discovery of a porphyria with excretion of coproporphyrin III by van den Bergh, Regniers, and Müller[15] in 1928, there has been a realization that sooner or later isomers of the other known porphyrins would be found to occur in nature. This was demonstrated for the uroporphyrins in 1935 by Waldenström,[16] and in the following year by Mertens,[17] and by Fischer and Libowitzky.[18] It has since been abundantly confirmed in human,[18, 19] bovine,[20] and squirrel[21] porphyria that both isomers are excreted simultaneously. Recently, Mertens[22] has re-

vealed indeed that in Petry feces the presence of coproporphyrin III in addition to coproporphyrin I had been overlooked, despite its presence in amounts as great as that of the more easily crystallized coproporphyrin I. This is of the greater importance since Grotepass[23] has reported that the two coproporphyrin isomers occur in equal amounts in normal urine. Recently, Fischer[24] has presented evidence that there is also a dualism of the blood pigments, in that, in addition to the previously identified protoporphyrin 9 obtainable from hemoglobin, there is also a small amount of protoporphyrin 2.

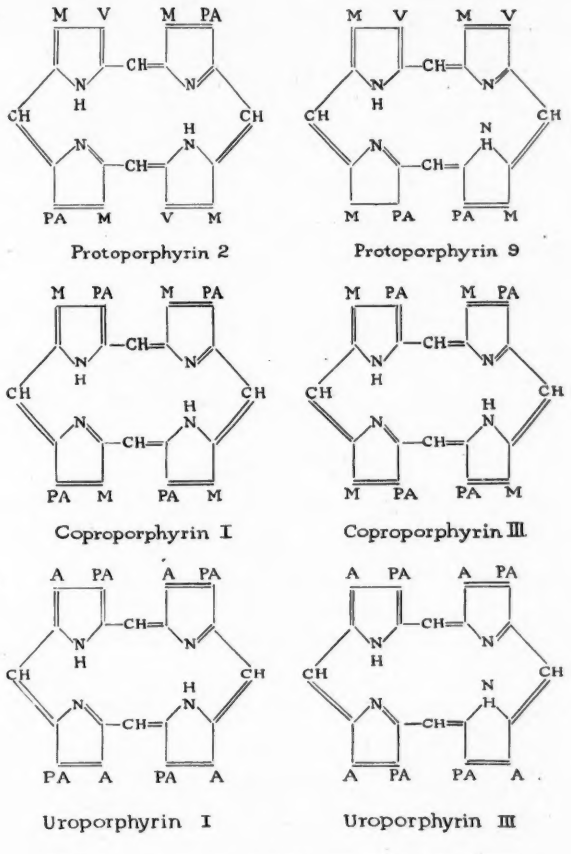


Figure 1

In 1936 Rimington,[25] appreciating the dualism of the porphyrins, proposed a schematic theory of enzyme action controlling not only the rate and type of porphyrins production, but also the formation of different porphyrins. His postulates, however, were too generalized for closer definition and merit no attention. In the same year, at the Baltimore meeting of the Southern Medical Society, Dobriner put forth a theory, published in 1937,[14a] that definitely proposed a chemical mechanism of porphyrinogenesis in keeping with known facts.

According to Dobriner, it is postulated that there are formed two pyrromethenes, A and B, which may combine in any of three possible ways: A + A

will give a type I porphyrin; B + B, a type II porphyrin; A + B, a type III porphyrin.

Type II porphyrins have not so far been discovered in biologic material, and Dobriner was led to suppose an enzymatic action which would act in such manner as to agree with actual facts. It will be noted that this ingenious theory still fails to explain the occurrence of three kinds of porphyrins.

By a glance at the formulas in Fig. I it will be apparent that these three kinds of porphyrins may be considered to be comprised of no more than three structural units; namely, 2-acetic acid, 3-propionic acid pyrrol; 2-methyl, 3-propionic acid pyrol, and 2-methyl, 3-vinyl pyrrol, of the structures in Fig. III.

Figure II

Pyrrol I Pyrrol II Pyrrol III

Figure III

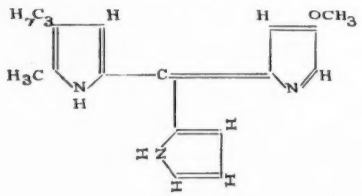


Figure IV

By simple decarboxylation of the acetic acid group pyrrol I is transformed into pyrrol II; dehydrogenation and decarboxylation of pyrrol II leads to pyrrol III. These reactions are well known in biologic processes, and it is not unimaginable that pyrrol I may actually be the primary building block of the naturally occurring porphyrins.

Such a pyrrol may well undergo an aldehyde synthesis to form a pyrromethene. Corwin and Andrews[26] have shown that in the aldehyde synthesis of pyrromethenes there is an intermediary formation of a tripyrrylmethane. It is of considerable interest that at least once in the experiments of Nature such a compound has been formed as a blind alley. This compound, prodigiosin, has been given the structure in Fig. IV.[27]

It may now be seen that if such a tripyrrylmethane were to be formed of the pyrrol I units, it may have the following form, which, on passing to the pyrromethenes, may lead to uroporphyrins I, II, and III:

Such a synthesis must be very rare, for it is seldom found, and it must occur only when the acetic acid group is protected from decarboxylation. Fischer

and Hofmann,[28] and Fischer and Müller[29] have shown that pyrrols of the type under discussion are unstable, losing carbon dioxide spontaneously at room temperatures.

The formation of an homologous trimethyl, tripropionic acid tripyrrylmethane might explain the formation of the coproporphyrins.

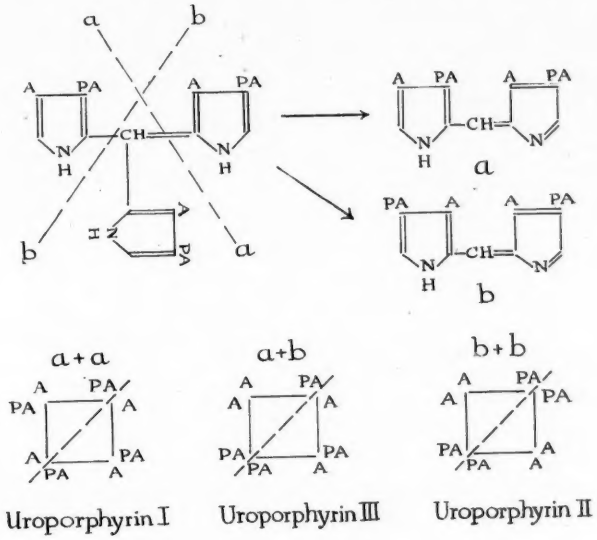


Figure V

However, the predominance of protoporphyrin as the apex of pyrrol metabolism indicates strongly that the extent of formation of pyrrol III must be large. The formation of protoporphyrin 2 and protoporphyrin 9 may then be accounted for by the intermediate formation and decomposition of a tripyrrylmethane according to the course indicated in Fig. VI.

In this theory, as well as in Dobriner's, there is expected the formation of some type II porphyrin (protoporphyrin 5). If the theory is correct, this will be found, unless there is some purely chemical reason, possibly associated with substituents on the 1 or 4 positions of the pyrrol rings, which would preclude it. It is, of course, likely that porphyrins are not formed without the action of enzymes, but of them nothing is known at present. However, the fact that uro- and coproporphyrins almost universally occur as their zinc complexes[6, 21, 30, 31] suggests that this metal may play a part in their formation.

Concerning the source of the proposed pyrrol I there is little to be said. Certainly it cannot arise from chlorophyll or any of its derivatives. Here it may be pointed out that there is no reason to believe that chlorophyll plays any part in the formation of animal porphyrins. Among the amino acids only tryptophan could conceivably be a precursor of pyrrol I, and this would require the improbable but possible opening of the 6-membered ring. This might occur by an oxidative scheme, such as is known with tyrosine and with quinone (Fig. VII).

However, as has been pointed out by Thomas,[32] there is no good evidence that tryptophan is essential in the formation of hemoglobin. Indeed, the work

of Fischer and his students, investigating the production of coproporphyrin by yeast,[33] and the study of Thomas[32] with the Harder's glands of rats as an indicator of protoporphyrin production, point to the probability that carbohydrate and simple amine metabolism is closely related to porphyrinogenesis.

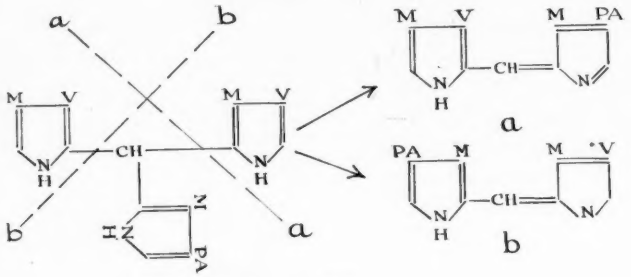


Figure VI

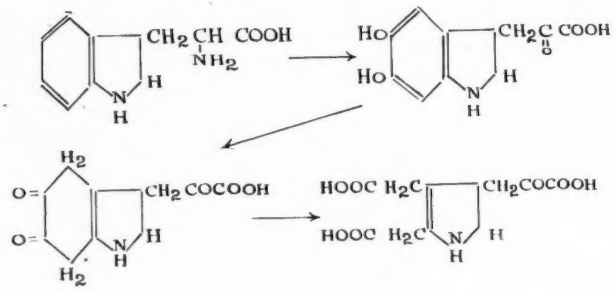


Figure VII

Finally, we may briefly consider the site of porphyrin formation. The rediscovery of cytochrome by Keilin in 1925, and the simultaneous demonstration of coproporphyrin synthesis by yeasts, reported by Fischer and his associates,[33] has been corroborated many times[30, 35] over. It is now plain that every unicellular organism is capable of forming its own porphyrins, and there is considerable evidence that this is indeed a general as well as an ancient cellular function. This formation appears to be characteristic of growing cells, so that in an adult animal the formation of porphyrins due to the majority of cells is probably slight. In view of the great activity of the erythropoietic system in the formation of hemoglobin, it is here that the evidences for cellular porphyrinogenesis must be sought. Indeed, it is here that the most straightforward evidence is found. Repeatedly, fluorescence characteristic of the three types of porphyrins has been observed in erythroblasts, gradually fading as the addition of iron and protein dampened it.[8, 13, 36] However, in porphyria, the frequent demonstration of zinc uroporphyrin,[31] as well as of other porphyrins[8, 13, 37] in the peripheral portion of liver lobules, suggests the possibility that in this condition cells other than erythropoietic may be active in porphyrinogenesis.

SUMMARY

A theory of porphyrinogenesis is proposed, based upon the postulated original formation of a 2-acetic acid, 3-propionic acid pyrrol, the products of which,

by passing through an aldehyde synthesis over a tripyrrylmethane, may form protoporphyrins 2 and 9, coproporphyrins I and III, and uroporphyrins I and III. The nature of the fundamental disturbance of metabolism in porphyria is suggested. The sources and sites of porphyrin formation are discussed.

REFERENCES

1. Günther, Hans: Die Hämatoporphyrie, Deutsches Arch. f. klin. Med. **105**: 89, 1911.
2 a. Fischer, Hans: Über das Urinporphyrin, Ztschr. f. physiol. Chem. **95**: 34, 1915.
   b. Über das Kotporphyrin, Ibid. **96**: 148, 1915.
   c. Über das Kotporphyrin, Ibid. **96**: 309, 1915.
   d. Die natürlichen Porphyrine und ihre Bedeutung für die Pathologie, Wien. klin. Wchnschr. **29**: 1027, 1916.
3. Turner, W. J., and Obermayer, M. E.: Studies on Porphyria. II, Arch. Dermat. and Syph. **37**: 549, 1938.
4. Waldenström, Jan: Studien über Porphyrie, Acta med. Scandinav. Suppl. 82, pp. 1-257, Stockholm, 1937.
5. Unpublished review of 230 cases by the author.
6. Turner, W. J.: Studies on Porphyria. III, Arch. Int. Med. **61**: 762, 1938.
7 a. Schumm, O.: Über das "Hämatoporphyrin" aus Harn und Knochen, Ztschr. f. physiol. Chem. **96**: 183, 1915.
   b. Über Porphyrinurie und natürliche Porphyrine, München. med. Wchnschr. **40**: 1300, 1923.
8. Borst, M. and Königsdorffer, H. Jr.: Untersuchungen über Porphyrie mit besonderer Berücksichtigung der porphyria congenita, Leipzig, 1929, S. Hirzel.
9. van den Bergh, A. A. Hijmans, Grotepass, W., and Revers, F. E.: Beitrag über das Porphyrin in Blut und Galle, Klin. Wchnschr. **11**: 1534, 1932.
10. Fischer, Hans, and Orth, H.: Die Chemie des Pyrrols, Band II. Leipzig, 1937, Akadem. Verlagsgesellschaft (in particular, page 480).
11. Schreus, H. T.: Ergebnisse und Probleme der Porphyrinforschung, Klin. Wchnschr. **13**: 121, 1934.
12. Carrié, C.: Die Porphyrine: Ihr Nachweis, ihre Physiologie und klinik, Leipzig, 1936, G. Thieme.
13. Vannotti, A.: Porphyrine und Porphyrinkrankheiten, Berlin, 1937, Julius Springer.
14 a. Dobrier, K., Strain, W. H., and Localio, S. A.: I. Quantitative Measurement of Coproporphyrin and Total Coproporphyrin I Excretion in Normals, Proc. Soc. Exper. Biol. & Med. **36**: 752, 1937.
   b. Dobriner, K., and others: II. Coproporphyrin I Metabolism and Hematopoietic Activity, Ibid. **36**: 755, 1937.
   c. Dobriner, K.: Excretion of Porphyrin by Dogs, Ibid. **36**: 757, 1937.
   d. Dobriner, K., and Barker, W. H.: Total Coproporphyrin I Excretion in Pernicious Anemia, Ibid. **36**: 864, 1937.
   e. Dobriner, K., and Rhoads, C. P.: The Excretion of Coproporphyrin I Following Hemorrhage in Dogs, J. Clin. Investigation **17**: 105, 1938.
   f. Dobriner, K., Rhoads, C. P., and Hummel, L. E.: The Excretion of Porphyrin in Refractory and Aplastic Anemia, Ibid. **17**: 125, 1938.
15. van den Bergh, A. A. Hijmans, Regniers, R., and Muller, W.: Ein Fall von kongenitaler Porphyrinurie mit Koproporphyrin im Harn und Stuhl, Arch. f. Verdauungskr. **42**: 302, 1928.
16 a. Waldenström, J.: Untersuchungen über Harnfarbstoffe, hauptsächlich Porphyrine, mittels der chromatographischen Analyse, Deutsches Arch. f. klin. Med. **178**: 38, 1935.
   b. Waldenström, J., Fink, H., and Hoerburger, W.: Über ein neues bei der akuten Porphyrie regelmässig vorkommendes Uroporphyrin, Ztschr. f. physiol. Chem. **233**: 1, 1935.
17. Mertens, E.: Über das Uroporphyrin bei akuter Porphyrie, Ztschr. f. physiol. Chem. **238**: 1, 1936.
18. Fischer, H., and Libowitzky, H.: Auftreten von Uro-bzw. Koproporphyrin I bei akuter Porphyrie, Ztschr. f. physiol. Chem. **241**: 220, 1936.
19. Dobriner, K.: Simultaneous Excretion of Coproporphyrin I and III in a Case of Chronic Porphyria, Proc. Soc. Exper. Biol. & Med. **35**: 175, 1936.
20. Rimington, C., Roets, G. C. S., and Fourie, P. J. J.: Quantitative Studies Upon Porphyrin Excretion in Bovine Congenital Porphyrinuria (Pink Tooth). No. I. Onderstepoort J. Vet. Sci. & Animal Ind. **10**: 421, 1938.
21. Unpublished studies of the author. See also Turner, W. J.: Studies on Porphyria I, Biol. Chem. **118**: 519, 1937.
22. Mertens, E.: Über das Vorkommen von Koproporphyrin III bei chronischer kongenitaler Porphyrie, Ztschr. f. physiol. Chem. **258**: 1, 1939.

23. Grotepass, W.: Zur Kenntnis der naturlichen Harnporphyrine, Ztschr. f. physiol. Chem. **253**: 276, 1938.
24. Fischer, H.: Zur Frage des Dualismus des Blutfarbstoffs, Ztschr. f. physiol. Chem. **259**: 1, 1939.
25. Rimington, C.: Some Cases of Congenita Porphyria in Cattle, Onderstepoort J. Vet. Sci. & Animal Ind. **7**: 567, 1936.
26 a. Corwin, A. H., and Andrews, J. S.: Studies in the Pyrrole Series, II. The Mechanism of the Aldehyde Synthesis of Dipyrrylmethenes, J. Am. Chem. Soc. **58**: 1086, 1936. b. III. The Relation of Tripyrrylmethane Cleavage to Methane Synthesis, Ibid. **59**: 1973, 1937.
27. Wrede, F., and Rothhaas, A.: Über das Prodigiosin, den roten Farbstoff des Bacillus prodigiosus, Ztschr. f. physiol. Chem. **226**: 95, 1934.
28. Fischer, H., and Hofmann, H. J.: Über die Konstitution des Uroporphyrins, Ztschr. f. physiol. Chem. **246**: 15, 1937.
29. Fischer, H., and Müller, A.: Synthese einer Tetramethyl-porphin-tetrasessigsäure. Ein Beitrag zum Konstitutionsproblem des Uroporphyrins, Ztschr. f. physiol. Chem. 246: 31, 1937.
30. Coulter, C. B., and Stone, F. M.: The Identification of the Porphyrin Compound Found in Cultures of C. diphtheriae and Mycobacteriae, Proc. Soc. Exper. Biol. & Med. **38**: 423, 1938.
31. Unpublished studies of the author on autopsy material from latent porphyria and personal communication from Dr. C. J. Watson.
32. Thomas, J.: Contribution à l'étude des porphyrines en biologie et en pathologie. I. Genèse du noyau tétrapyrrolique, Bull. Soc. Chim. Biol. **20**: 471, 1938.
33 a. Fischer, H., and Hilger, J.: Zur Kenntnis der natürlichen Porphyrine. X. Blutfarbstoff in der Hefe, Nachweis von Porphyrin in Pflanzen, Ztschr. f. physiol. Chem. **138**: 288, 1924.
b. Fischer, H., and Fink, H.: Über Koproporphyrinsynthese durch Hefe and ihre Beeinflussung, Ibid. **150**: 243, 1925.
34. Keilin, D.: On Cytochrome, a Respiratory Pigment, Common to Animals, Yeast, and Higher Plants, Proc. Roy. Soc., London, s. B. **98**: 312, 1925.
35. Jakob, A.: Über den Abbau von Blutfarbstoff zu Porphyrinen durch Reinkulturen von Bakterien und über eine neue biologische Synthese von Koproporphyrin III, Klin. Wchnschr. **18**: 1024, 1939.
36. Emminger, E., and Battistini, G.: Fluorescenzmikroskopische Untersuchungen an bleivergifteten Kaninchen mit und ohne Calciumzufuhr, Virchows Arch. f. path. Anat. **290**: 492, 1933.
37. Emminger, E.: Fluorescenzmikroskopische Untersuchungen bei einem Fall von Schlafmittelvergiftung, Klin. Wchnschr. **12**: 1840, 1933.

STUDIES ON THE INTRAVENOUS INJECTION OF COLLOIDS*

II. Effects of Gum Acacia on Certain Functions of the Liver With a Note on Its Effects on the Production of Immune Bodies

W. Knowlton Hall,† Ph.D., New Orleans, La., and R. B. Gibson, Ph.D., and L. A. Weed, M.D., Ph.D., Iowa City, Iowa

ANDERSCH and Gibson[1] in 1934 demonstrated in a series of experimental observations that a large part of the gum acacia injected intravenously in rabbits and in dogs was not excreted, as had been generally assumed, but was deposited in the hepatic cells. An average of 30 and 33 per

*From the Pathological Chemistry Laboratory, University Hospitals, the State University of Iowa, and the Department of Biochemistry, School of Medicine, Louisiana State University, New Orleans.

†This report includes a condensation of a portion of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry, in the Graduate College of the State University of Iowa, July, 1936. A preliminary report appeared in Proc. Soc. Exper. Biol. & Med. **38**: 46, 1938.

Received for publication, November 20, 1939.

cent, respectively, was found in the livers of two dogs studied, and an average of 50 per cent in the livers of rabbits. Histologic examination of the liver tissue, and marked changes in the composition of the bile, especially of the bile pigment excreted by the dogs under observation, led these authors to conclude that intravenous injections of gum acacia were likely to cause marked liver damage. In the same contribution they reported a fatal clinical case of nephrosis in which this form of therapy had been employed and in which 43 per cent of the injected acacia was found in the liver. The liver cells presented the same characteristic vacuolated appearance noted in the liver cells of the experimental animals.

In the following year Dick, Warweg, and Andersch[2] extended these clinical and experimental observations and concluded that the intravenous use of gum acacia in patients with nephrosis was likely to be attended with harmful effects.

In 1937 Studdiford[3] called attention to the extreme danger of employing intravenous injections of gum acacia in patients with already damaged livers, such as are likely to be found in the toxemias of pregnancy and similar disorders. This form of therapy, he pointed out, might interfere with the gaseous exchanges of the red blood cells, and if carried to a marked degree, might produce severe or fatal anoxemia, especially if faulty solutions were employed. He also produced evidence to show that such solutions might cause "conglutination of the red cells, with resultant capillary blockage followed by edema and hemorrhage."

The purpose of the present contribution is twofold: to study liver function by various tests after the experimental intravenous injection of acacia, and to observe the possible effects of this method of treatment on the production of immune bodies. The general procedures employed in the handling of the experimental animals and the injection of the gum acacia, together with the methods of analysis used for acacia, are set forth in detail by Andersch and Gibson.[1]

STUDIES IN LIVER FUNCTION

Glucose and Galactose Tolerance Tests.—Glucose and galactose tolerance tests were performed on normal dogs after they had fasted eighteen hours or more. Blood for a control sugar determination[4] was taken from the marginal vein of the ear, and other samples were taken every half hour for a two-hour period following the administration by stomach tube of 1.5 Gm. of sugar per kilogram of body weight (Table I). The blood galactose,[5] as well as the total sugar, was determined subsequently in two more dogs in the course of the galactose tolerance tests. All the dogs were then given from three to seven intravenous injections of gum acacia (totaling 4.4 to 10.7 Gm. per kilogram of body weight) over periods varying from three days to four weeks. Several days after the last acacia injection the glucose and galactose tolerance tests were repeated.

The control glucose curves obtained in this study are similar to those obtained by Bodansky[6] after the oral administration of sugar, and by Soskin, Allweiss, and Cohn[7] after its intravenous administration. The curves ob-

tained after the injections of acacia apparently correspond with the glucose tolerance curves obtained by Soskin and Mirsky[8] in severe liver intoxications.

The galactose blood clearance controls agree with the curves obtained on normal dogs by Bodansky.[6] A marked diminution in galactose tolerance was observed after the injection of acacia.

TABLE I

EFFECT OF INTRAVENOUS INJECTIONS OF ACACIA UPON TOLERANCE OF DOGS TO GLUCOSE AND GALACTOSE

DOG NO.	ACACIA TOTAL GM./KG.	GLUCOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT OF GLUCOSE							GALACTOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT AS GLUCOSE						
		0.0	0.5	1.0	1.5	2.0	2.5	3.0	0.0	0.5	1.0	1.5	2.0	2.5	3.0
1	0	100	147	107		81			92	157	145	131	129		
	4.4	120	157	157	140	98			98	200	236	212	162		
2	0	81	118	107	82	79			81	162	165	192	173		
	7.5	69	165	179	138	79			90	131	189	231	175		
4	0	50	150	122	80	78			87	135	122	96	85		
	9.0	80	170	147	90	95			90	142	186	157	102		
5	0	92	179	173	90	74			120	167	212	150	142		
	10.7	96	217	212	165	96			102	192	226	212	167		
6	0								79	167	186	159	129		
	8.4								86	186	226	182	138		
		GALACTOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT OF GALACTOSE													
10	0		80*	112	100	68			80	143	170	156	139		
	8.5		59	73	97	81	45		90	142	155	172	138	125	
11	0		71*	91	122	109	31		91	154	167	200	183	107	
	6.8		101	161	164	151	66	35	85	160	221	225	223	133	113

*The total sugar values obtained in these tests are reported on the same line on the right side of the table.

Tetraiodophenolphthalein and Bilirubin Tests.—Dogs were tested by a modification of the sodium tetraiodophenolphthalein liver function procedure of Graham.[9] Ninety seconds after the injection into the jugular vein of 25 mg. per kilogram body weight of tetraiodophenolphthalein (isoiodeikon), a sample of blood was withdrawn from the vein on the opposite side and oxalated. Additional samples of blood were obtained fifteen, thirty, and sixty minutes later. One cubic centimeter of plasma from each specimen was made alkaline with 2 drops of 10 per cent sodium hydroxide, 10 c.c. of ethyl alcohol were added, the contents of each tube were mixed, placed in the icebox overnight, and centrifuged in the morning. The test of the first blood sample was used as a standard for the colorimetric determination of the dye in the subsequent specimens.

Observations on four dogs revealed no significant changes in the ability of the liver to excrete the dye before and after injections of gum acacia; in two other dogs there was apparently a slight reduction.

For the bilirubin test the method suggested by Soffer[10] was employed. One milligram of bilirubin per kilogram of body weight was given intravenously to two normal dogs and to two dogs which had had had intravenous

injections of acacia (Table II). Samples of blood were withdrawn before injection of the pigment, and again five minutes and four hours afterward. The plasma bilirubin in the specimens was determined quantitatively by the Gibson and Goodrich[11] modification of the van den Bergh procedure. The Soffer method, which was suggested for clinical use, gave a rather low degree of bilirubinemia. We therefore repeated the test, using the procedure suggested by Dragstedt and Mills.[12] Ten milligrams of bilirubin per kilogram of body weight were injected intravenously, and blood samples were withdrawn before the injection, and five minutes, thirty minutes, one hour, and two hours afterward. In none of the four dogs used was there any alteration in the rate of removal of the injected bilirubin.

TABLE II

BILIRUBIN LIVER FUNCTION TEST ON NORMAL DOGS AND DOGS AFTER ACACIA INJECTIONS

DOG NO.	WEIGHT (KG.)	ACACIA INJECTED (GM.)	BILIRUBIN INJECTED (MG.)	VAN DEN BERGH—MG. BILIRUBIN TIME IN MINUTES						
				0	2.5	5	30	60	120	240
3	9.5	0	10.0	0.2		1.2				0.4
5	8.7	96.6	8.7	0.6	1.9	1.3				0.7
6	8.0	67.5	8.0	0.5		0.7				0.6
7	9.2	0	9.2	0.3	1.9	2.0				0.6
5	8.7	96.6	87.0	0.2		15.3	5.0	2.9	2.1	
7	9.2	0	92.0	0.3		15.0	5.3	2.8	2.5	
8	5.5	0	55.0	0.2		7.0	3.7	1.4	1.0	
8	5.5	41.4	55.0	0.2		4.0	1.8	1.0	0.9	
9	5.0	0	50.0	0.3		6.5	3.9	1.4	0.9	
9	5.0	45.0	50.0	0.4		6.8	2.7	0.9	0.9	

TABLE III

EFFECT OF INTRAVENOUS ACACIA INJECTIONS ON PLASMA PROTEIN LEVEL

DOG NO.	WEIGHT (KG.)	PLASMA PROTEIN IN GRAMS PER CENT								REMARKS
		ALBUMIN		GLOBULIN		FIBRINOGEN		TOTAL PROTEIN		
		BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	
2	9.7	2.94	2.54	1.66	1.86	0.38	0.14	4.98	4.54	82.5 Gm. acacia blood drawn 4 days after last acacia injection
4	7.5	2.52	2.11	2.74	2.58	0.41	0.24	5.67	4.93	67.5 Gm. acacia blood drawn 3 days after last acacia injection
5	8.7	2.44	1.72	1.96	1.10	0.32	0.04	4.70	2.86	96.6 Gm. acacia blood drawn 4 days after last acacia injection
6	8.0	2.74*	2.20	2.03*	1.80	0.37*	0.21	5.14*	4.20	67.5 Gm. acacia blood drawn 5 days after last acacia injection

*This sample of blood was drawn a few minutes after the first acacia injection.

Plasma Protein Determinations.—Plasma protein determinations were made by the colorimetric method of Andersch and Gibson,[13] modified by washing the fibrinogen precipitate and substituting the tyrosine factors

of Greenberg and Mirolubova[14] in the calculation. Observations were made before, during, and after the intravenous injection of acacia (Table IV). Albumin-globulin ratios on three dogs were 1.11, 0.92, and 1.24, respectively, before the injections, and 1.36, 0.82, and 1.56, respectively, after the injections. Finbrinogen was markedly reduced. In one dog it fell to 0.04. In another it fell to 0.14 and rose only to 0.25 per cent twenty-one days after the last acacia injection had been given. These values are to be compared with the average normal of 0.39 per cent set up by Foster and Whipple,[15] who consider the possible normal range from 0.306 to 0.506.

TABLE IV

EFFECTS OF INTRAVENOUS INJECTIONS OF ACACIA ON CERTAIN PROPERTIES OF THE BLOOD

	DOG 2		DOG 4		DOG 5		DOG 6	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
Bleeding time (minutes)	4.5	10.5	4.5	6.0	3.0	5.0	5.5	4.0
Coagulation time (minutes)								
Capillary	3.5	2.5	2.5	3.0	3.0	3.0	2.0	7.0
Venous	4.0	3.0	3.0	5.0	3.5	4.5	4.0	9.0
Fragility (per cent NaCl)	0.34-0.44	0.32-0.46	0.32-0.44	0.38-0.48	0.36-0.44	0.36-0.48	0.28-0.46	0.40-0.50
Platelets (per cent)	0.98	0.24	1.45	1.26	0.83	1.90	0.31	1.43
Hematocrit (per cent)	53.5	46.0	37.0	38.0	46.0	45.5	49.8	33.2
Hemoglobin—Newcomer (grams)	13.2	11.3	9.4	9.3	11.0	10.4	12.1	11.3
Reticulocytes (per cent)	0.8	0.4	0.4	1.2	0.8	0.5	1.2	0.6
Clot retractility (4 hours)	2+	No clot	4+	4+	4+	4+	4+	3+

Hematologic Studies.—Because of the demonstrated low plasma fibrinogen values after the administration of acacia, as well as the possibility of changes in other factors essential in blood clotting, several hematologic tests ordinarily used in blood dyscrasias were employed (Table IV). The methods are described in detail by Baldridge and Fowler.[16] Some difficulty was encountered in determinations of the bleeding time because of the unequal vascularity of different portions of the dog's ear, as well as the tendency toward mechanical closing of the stab wound on account of the thick skin. Variation in the sedimentation rates of the platelets and erythrocytes also interfered with the platelet determination, which was done by the thrombocytocrit method of Van Allen.

In spite of these difficulties, it was possible to show that the injection of acacia led to a prolongation of the bleeding time in three or four dogs studied. The blood of one dog, drawn for a study of clot retraction, failed to coagulate in four hours. The clot formed from the blood of another dog was a fragile, fibrinous network which completely failed to enmesh the red blood cells. The other tests, except for a definite decrease in hemoglobin in all the dogs observed, were not consistent or significant.

DISCUSSION

It is quite generally accepted at this time that the liver is concerned in determining the degree of glucose tolerance, provided that the insulin supply of the animal is not impaired.[17] The blood glucose curves obtained after the injection of acacia obviously seem to indicate diminished tolerance. Galactose is

almost specifically utilized by the liver. The galactose curves are more decisive, and the diminution of galactose tolerance noted after the injection of acacia undoubtedly indicates liver damage. The impairment of glycogenic function after acacia injection is also borne out by post-mortem observations on liver glycogen made on three dogs. The livers contained respectively 6.6, 7.7, and 6.9 per cent of glycogen, an average of 7.1 per cent against the normal value of 15 per cent to be expected in dogs fed, as these were, a high carbohydrate diet. Bollman and Mann[18] have observed that dogs with 80 per cent of the liver removed are still able to take care normally of glucose and galactose.

The figures which Soffer[10] gives in an extensive review (including his own work) show that an abnormal retention of bilirubin is more closely correlated with the degree of liver damage than are the bromsulphalein or the levulose tolerance tests of liver function. We found no significant alteration in the rate of removal of injected bilirubin before and after the injection of gum acacia, and only a slight variation in two dogs in the excretion of tetraiodophenolphthalein. It seems logical to conclude, therefore, that this special function of the liver is not affected by the injection of acacia.

The experimental studies of Whipple and his co-workers[19-21] point to the liver as the source of serum albumin and globulin. A number of studies by others[22-26] are concerned with the effect of severe liver disease on the plasma proteins, particularly papers by Snell[25] and by Myers and Keefer.[26] These bear out Snell's conclusion that in advanced chronic hepatic lesions there is "a moderate reduction in the total serum proteins, the diminution occurring chiefly in the albumin fraction." Dick, Warweg, and Andersch[2] have shown that repeated injections of acacia in children with nephrosis resulted in a reduction of blood serum protein, chiefly albumin. They also found that daily injections of acacia into dogs caused a sharp drop in the serum albumin and a less marked fall in the globulin, both values returning to normal when the injections were discontinued. Our own observations are in accord with these results, as are the more recent studies of Heckel and his associates.[27]

Foster and Whipple[28] have shown that fibrinogen is formed exclusively by the liver. Low values may be found in hepatic disease, though the picture may be complicated by the stimulating effects of infection on fibrinogen production. The effect on fibrinogen levels of various clinical and experimental conditions involving the liver has been reviewed by Starlinger and Winands[29] and by Ham and Curtis.[30] It may be said that complete extirpation of the liver in dogs[31] and in rabbits[32] results in a rapid fall in plasma fibrinogen, in some experiments amounting to 50 per cent in twenty-four hours; removal of as much as 70 per cent of hepatic tissue, on the other hand, has no effect on the fibrinogen level.

Foster and Whipple,[33] who found lowered blood fibrin values after the injection of acacia, ascribed the reduction to dilution of the blood. The possible influence of gum-saline solutions on blood coagulation is mentioned by Takata[34] and others. Dieckmann,[35] who tried gum acacia injections in the treatment of eclampsia, specifically warns that this form of therapy may cause a pronounced drop in blood fibrin. The decrease in hemoglobin, the increased bleeding time, and the low fibrinogen values noted in our experimental animals after the injec-

TABLE V

PRODUCTION OF IMMUNE BODIES IN NORMAL DOGS AND IN DOGS WHICH HAD HAD ACACIA INJECTIONS

DOG NO.	WEIGHT (KG.)	GRAMS OF ACACIA PER KG.	TITER OF AGGLUTININ TO VIBRIO CHOLERA		TITER OF PRECIPITIN TO VIBRIO CHOLERA		TITER OF AGGLUTININ TO ERYTHROCYTES		TITER OF PRECIPITIN TO HEMOLYZED ERYTHROCYTES		TITER OF HEMOLYSIN	
			BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION
2	9.7	7.3	0	1/2,560	0	1/12,800	0	1/640	0	1/204,800	1/4	1/50
3	9.5	0	0	1/5,120	0	1/12,800	0	1/12,800	0	1/204,800	1/4	1/200
5*	8.7	10.7	0	1/320	1/200	1/200	0	1/2,560	1/200	1/1,600	0	1/40
6*	8.0	8.4	0	1/320	0	0	0	1/2,560	0	1/1,600	1/10	1/40
7*	9.2	0	0	1/1,280	1/200	0	0	1/2,560	0	1/3,200	1/10	1/40

*The titration of antibodies "before immunization" with Dogs 5, 6, and 7 was done on blood drawn three days after the first injection of antigen. Dogs 3 and 7 are normal animals.

tion of gum acacia are undoubtedly significant and would seem to indicate a rather severe degree of liver damage. Additional confirmation is found in the death of one dog from a postoperative hemorrhage after several injections of gum acacia.

EFFECT OF COLLOIDS ON THE PRODUCTION OF IMMUNE BODIES

The injection of various colloids has been shown to result in a marked reduction in the ability of the animal to produce immune bodies.[36] These studies have led to an association of the reticulo-endothelial system with antibody production. However, the hepatic cells of the liver have never been definitely excluded from a role in antibody formation. The plasma proteins which carry the antibodies of the blood have their origin in the liver, and the ability of the organism to produce these proteins is impaired by the acacia which is deposited in the hepatic cells. Acacia itself is at most only a very mild antigen.[37, 38]

In order to determine the effect of acacia injections upon the ability of the dog to produce immune bodies, five animals, of approximately equal weight, were used. Three of them (Nos. 2, 5, and 6) had had intravenous gum injections and had been tested by various liver function tests (Tables I, III, and IV). The other two (Nos. 3 and 7) were used as controls.

Two antigens were used. The first was a 5 per cent suspension of pooled washed human erythrocytes. The second was a vaccine of *Vibrio cholera* made from whole and ground organisms which had been killed by phenyl mercuric nitrate, 0.12 per cent; the cholera was strain No. 23 of the American Type Culture Collection. Five cubic centimeters of the suspension of human red blood cells were injected intravenously every three days until five injections had been given; 0.1 c.c. of the cholera vaccine was injected intravenously with the erythrocyte suspension for three days, and 0.2 c.c. for the last two days. Eight days after the final injection of the antigens the animals were bled and the titer of agglutinins, precipitins, and bacteriolysins or hemolysins was determined. Samples of blood drawn before immunization were also checked for the presence of antibodies.

The presence of cytolysins for *Vibrio cholera* could not be demonstrated in either the normal or the immune sera. Hemolysin titrations were not very satisfactory, and the titers produced were low. Differences between the titers of antibody produced by normal dogs and those receiving acacia were not significant, and the data all indicate that gum acacia has no effect upon the production of immune bodies (Table V). This lack of effect is in marked contrast to the reduction of antibody formation likely to result when colloids which definitely block the reticulo-endothelial system are injected.

SUMMARY AND CONCLUSIONS

1. Repeated intravenous injections of gum acacia solution in dogs resulted in evident damage for carbohydrate and serum protein metabolism functions of the liver, as evidenced by changes in the glucose and galactose blood sugar tolerance curves and determination of the plasma proteins.

2. The blood clearance of injected tetraiodophenolphthalein and bilirubin was not significantly affected.

3. An alteration in the clotting mechanism of the blood was noted after acacia injections, the fall in fibrinogen content being particularly marked. The bleeding time was prolonged, and the formation of small, fragile fibrous clots clearly indicated a defect presumably associated with the lowered fibrinogen content.

4. The production of agglutinin, hemolysin, bacteriolysin, and precipitin was not influenced by acacia administration. There was neither an effect on the hepatic cells nor was there a blocking of the reticulo-endothelial system as produced by certain colloidal substances.

We are grateful to the Eli Lilly Co. for donating part of the ampoules of acacia which were used in these experiments.

REFERENCES

1. Andersch, N., and Gibson, R. B.: Studies on the Effects of Intravenous Injections of Colloids. I. Deposition of Acacia in the Liver and Other Organs and Its Excretion in Urine and Bile, J. Pharmacol. & Exper. Therap. **52**: 390, 1934.
2. Dick, M. W., Warweg, E., and Andersch, M.: Acacia in the Treatment of Nephrosis, J. A. M. A. **105**: 654, 1935.
3. Studdiford, W. E.: Severe and Fatal Reactions Following the Intravenous Use of Gum Acacia Glucose Infusions, Surg. Gynec. & Obst. **64**: 772, 1937.
4. Gibson, R. B.: The Micro-Determination of Blood Sugar, Proc. Soc. Exper. Biol. & Med. **27**: 480, 1930.
5. Roe, J. H., and Schwartzman, A. S.: Galactose Tolerance of Normal and Diabetic Subjects, and the Effect of Insulin Upon Galactose Metabolism, J. Biol. Chem. **96**: 717, 1932.
6. Bodansky, M.: Fructose, Glucose, and Galactose Tolerance in Dogs, J. Biol. Chem. **56**: 387, 1923.
7. Soskin, S., Allweiss, M. D., and Cohn, D. J.: Influence of the Pancreas and the Liver Upon the Dextrose Tolerance Curve, Am. J. Physiol. **109**: 155, 1934.
8. Soskin, S., and Mirsky, I. A.: The Influence of Progressive Toxemic Liver Damage Upon the Dextrose Tolerance Curve, Am. J. Physiol. **112**: 649, 1935.
9. Graham, E. A.: Estimating the Risk of Operations on the Biliary Tract by Testing the Excretory Function of the Liver, Radiology **21**: 191, 1933.
10. Soffer, L. J.: Present Day Status of Liver Function Tests, Medicine **14**: 185, 1935.
11. Gibson, R. B., and Goodrich, G. E.: Determination of Plasma Bilirubin: A Modified van den Bergh Procedure, Proc. Soc. Exper. Biol. & Med. **31**: 413, 1934.
12. Dragstedt, C. A., and Mills, M. A.: Bilirubinemia and Bromsulphalein Retention, Proc. Soc. Exper. Biol. & Med. **34**: 467, 1936.
13. Andersch, M., and Gibson, R. B.: The Colorimetric Determinations of Plasma Proteins, J. Lab. & Clin. Med. **18**: 816, 1933.
14. Greenberg, D. M., and Mirolubova, T. N.: Modifications in the Colorimetric Determination of the Plasma Proteins by the Folin Phenol Reagent, J. Lab. & Clin. Med. **21**: 431, 1936.
15. Foster, D. P., and Whipple, G. H.: Blood Fibrin Studies. II. Normal Fibrin Values and the Influence of Diet, Am. J. Physiol. **58**: 379, 1922.
16. Baldridge, C. W., and Fowler, W. M.: Notes on Hematology, Iowa City, Iowa, University Printing Service, pp. 11-12, 22-25.
17. Chaikoff, I. L.: Carbohydrate Metabolism, Ann. Rev. Biochem. **5**: 205, 1936.
18. Bollman, J. L., and Mann, F. C.: Alterations in Hepatic Function Produced by Experimental Hepatic Lesions, Ann. Int. Med. **9**: 617, 1935.
19. Kerr, W. J., Hurwitz, S. H., and Whipple, G. H.: Regeneration of Blood Proteins, Am. J. Physiol. **47**: 379, 1918.
20. Knutti, R. E., Erickson, C. C., Madden, S. C., Rekers, P. E., and Whipple, G. H.: Liver Function and Blood Plasma Protein Formation. Normal and Eck Fistula Dogs, J. Exper. Med. **65**: 455, 1937.
21. Holman, R. L., Mahoney, E. B., and Whipple, G. H.: Blood Plasma Protein Regeneration Controlled by Diet. I. Liver and Casein as Potent Diet Factors, J. Exper. Med. **59**: 251, 1934.
22. Kaunitz, H.: Serumeiweisskörper bei Leberkrankheiten, Wien. klin. Wchnschr. **48**: 1349, 1935.
23. Foley, F. F., Keeton, R. W., Kendrick, A. B., and Darling, D.: Alterations in Serum Proteins as an Index of Liver Failure, Proc. Soc. Exper. Biol. & Med. **33**: 430, 1935.

24. Starlinger, W., and Winands, E.: Über das Verteilungsverhältnis der zirkulierenden Eiweisskörper im Verlaufe krankhafter Züstände (Spontan und Reizschwankungen bei Gesunden und Kranken, eitrige und seröse Ergüsse, Zusammenfassung der Ergebnisse), Ztschr. f. d. ges. exper. Med. **60**: 208, 1928.
25. Snell, A. M.: Changes in the Proteins of Blood in Hepatic Disease, Proc. Staff Meet., Mayo Clin. **10**: 489, 1935.
26. Myers, W. K., and Keefer, C. S.: Relation of Plasma Proteins to Ascites and Edema in Cirrhosis of the Liver, Arch. Int. Med. **55**: 349, 1935.
27. Heckel, G. P., Erickson, C. C., Yuile, C. L., and Knutti, R. E.: Blood Plasma Proteins as Influenced by Intravenous Injection of Gum Acacia, J. Exper. Med. **67**: 345, 1938.
28. Foster, D. P., and Whipple, G. H.: Blood Fibrin Studies. IV. Fibrin Values Influenced by Cell Injury, Inflammation, Intoxication, Liver Injury and the Eck Fistula. Notes Concerning the Origin of Fibrinogen in the Body, Am. J. Physiol. **58**: 407, 1922.
29. Starlinger, W., and Winands, E.: Über das Verteilungsverhältnis der zirkulierenden Eiweisskörper im Verlaufe krankhafter Zustände (Erkrankungen der blut bildenden und zerstörenden Organe und der Leber, bösartige Geschwülste, Leukämie, Granulom, Magendarmgeschwür, Gastroenteritis, Diabetes, und andere Erkrankungen der innersekretorischen Organe), Ztschr. f. d. ges. exper. Med. **60**: 185, 1928.
30. Ham, T. H., and Curtis, F. C.: Plasma Fibrinogen Response in Man. Influence of the Nutritional State, Induced Hyperpyrexia, Infectious Disease and Liver Damage, Medicine **17**: 413, 1938.
31. Jones, T. B., and Smith, H. P.: Blood Fibrinogen Level in Hepatectomized Dogs and an Outline of a Method for the Quantitative Determination of Fibrinogen, Am. J. Physiol. **94**: 144, 1930.
32. Drury, D. R., and McMaster, P. D.: The Liver as the Source of Fibrinogen, J. Exper. Med. **50**: 569, 1929.
33. Foster, D. P., and Whipple, G. H.: Blood Fibrin Studies. III. Fibrin Values Influenced by Transfusion, Hemorrhage, Plasma Depletion and Blood Pressure Changes, Am. J. Physiol. **58**: 393, 1922.
34. Takata, M.: The Influence of Intravenous Injections of Ringer-Locke's Solution Containing Gum Arabic on Coagulability of Blood, Folia pharmacol. japon. **8**: 3, 1929.
35. Dieckmann, W. J.: Gum Acacia in the Treatment of Eclampsia. A Preliminary Report, Am. J. Obst. & Gynec. **22**: 351, 1931.
36. Topley, W. W. C.: An Outline of Immunity, Baltimore, 1933, William Wood & Co.
37. Maytum, C. K., and McGath, T. B.: Sensitivity to Acacia, J. A. M. A. **99**: 2251, 1932.
38. Spielman, A. D., and Baldwin, H. S.: Atopy to Acacia (Gum Arabic), J. A. M. A. **101**: 444, 1933.

BLOOD SERUM MAGNESIUM IN BRONCHIAL ASTHMA AND ITS TREATMENT BY THE ADMINISTRATION OF MAGNESIUM SULFATE*

VICTOR G. HAURY, M.D., PHILADELPHIA, PA.

A NUMBER of investigators have recently shown that magnesium salts will protect animals against anaphylactic shock. Delbet and Palios[1] have found that a sensitized guinea pig may be protected against the provocative injection of the specific antigen by parenteral injections of magnesium salts. Lumiére and Monchal[2] have also found that sensitized rabbits may be protected by one or more daily injections of 0.1 Gm. per kg. of magnesium thiosulfate. They observed that such injections of the thiosulfate produced a 50 to 100 per cent increase in blood cholesterol and concluded that the antishock action of this salt was due entirely to the cholesterolemia it produced. I[3] have observed, however, that if the serum magnesium of sensitized guinea pigs is increased to about 8 mg. per 100 c.c. immediately before the provocative injection of the antigen is made, anaphylactic shock may be prevented. This would seem to indicate that hypermagnesemia rather than a cholesterolemia is the protective agent. If hypermagnesemia will prevent anaphylactic shock, we may hypothesize that hypomagnesemia may make one more sensitive to, or even incite an anaphylactic reaction.

The similarity between the bronchial spasms in anaphylactic shock and bronchial asthma is now generally conceded. The possibility that a hypomagnesemia may be an etiologic factor in bronchial asthma naturally suggested itself. The findings of Rosella and Plá[4] that intravenous injection of magnesium sulfate relieved a case of asthma which was unaffected by the common remedial agents, adds an additional premise to this theory.

The present investigation was undertaken to determine, if possible, if patients suffering from bronchial asthma have a concomitant hypomagnesemia.

METHOD

Sixty-six asthmatic patients were chosen at random as admitted to the Allergy Clinic and the Medical Wards of the Jefferson College Hospital.†

About 10 c.c. of blood were collected from each patient from the median basilic vein. Serum magnesium determinations were made by the Titan yellow method described elsewhere.[5] In the hospitalized patients a series of determi-

*From the Department of Pharmacology, Jefferson Medical College, Philadelphia.

This investigation was made possible through a grant from Parke, Davis & Co. to Jefferson Medical College for research in science.

Received for publication, October 27, 1939.

†I wish to thank Doctors J. A. Clark and H. A. Reimann, Chiefs of the Allergy and Medical Services, respectively, of the Jefferson College Hospital, for their cooperation in making this investigation possible.

nations were made at daily intervals. The serum magnesium values of 40 normal medical students were previously ascertained. As the ingestion of food has very little influence on the concentration of serum magnesium, no effort was made to obtain blood at any specified period in relation to meals. All determinations were made in duplicate.

RESULTS

Normal Persons.—Forty determinations were made, using blood obtained from students in the medical school. The values obtained varied between 1.74 and 3.10 mg. per 100 c.c. serum, with a mean value of 2.33 mg. These figures are in accordance with those found by Becher,[6] Bomskov,[7] Wacker and Fahrig,[8] Walker and Walker,[9] and Hirschfelder and myself.[10]

Serum magnesium values below 1.7 mg. per 100 c.c. serum are, therefore, considered as being definitely below normal. Values above 3.1 mg. are considered as being high.

Asthmatic Subjects.—The serum magnesium of the 66 individuals suffering from bronchial asthma varied between 1.1 and 3.15 mg. per 100 c.c., with a mean value of 2.13 mg. Of the entire group of 66 individuals 16 (24.2 per cent) had a low serum magnesium, varying from 1.1 to 1.7 mg. per 100 c.c., with a mean value of 1.52 mg. Twenty-six of the patients examined were suffering an acute attack of asthma at the time the blood samples were taken. In these the serum magnesium values varied between 1.1 and 3.1 mg per 100 c.c., with a mean value of 1.82. The remaining 40 individuals were free of asthma at the time the blood was analyzed. In these the mean magnesium value was 2.32 mg. per 100 c.c. serum (see Table I).

Upon further analysis of the data, one finds that of the 26 patients having acute asthmatic spasms, 13 (50 per cent) had a concomitant hypomagnesemia. In this latter group the serum magnesium varied between 1.10 and 1.70 mg., with a mean of 1.50 mg. per 100 c.c. The serum magnesium of the 13 remaining cases was entirely within normal range.

The Effect of Injections of Magnesium Sulfate.—Two patients suffering from severe asthma were treated with intravenous and intramuscular injections of magnesium sulfate. The results obtained were essentially the same as those reported by Rosella and Plá.[4]

Two white male patients, aged 39 and 56 years, respectively, hospitalized at the Jefferson Medical College Hospital, were suffering with acute bronchial asthma. Both patients had a low serum magnesium, averaging 1.53 and 1.60 mg. per 100 c.c., respectively, over a four-day period. Epinephrine administration was required every four to six hours, but on several occasions this drug was ineffective. This form of treatment was discontinued, and at the height of the asthmatic paroxysms 20 c.c. of 10 per cent magnesium sulfate were injected slowly intravenously and 4 c.c. of the 50 per cent solution were given deep intramuscularly. Both patients were relieved immediately and remained free of asthma for eighteen and twenty-eight hours, respectively. Following the reappearance of the bronchial spasms, epinephrine therapy was again instituted, requiring as before, an injection every four to six hours.

TABLE I

MAGNESIUM CONTENT OF THE SERUM OF ASTHMATIC AND NORMAL INDIVIDUALS

	NO. OF INDIVIDUALS	MG. MAGNESIUM PER 100 C.C. SERUM														
		1.1 TO 1.2	1.2 TO 1.3	1.3 TO 1.4	1.4 TO 1.5	1.5 TO 1.6	1.6 TO 1.7	*	1.7 TO 1.8	1.8 TO 1.9	1.9 TO 2.0	2.0 TO 2.2	2.2 TO 2.4	2.4 TO 2.6	2.6 TO 2.8	2.8 TO 3.0	ABOVE 3.0
During attack	26	2	0	2	1	3	5		2	5	1	2	0	1	1	0	1
4 to 24 hours after last attack	3	–	–	–	–	–	–		–	–	–	–	2	–	–	–	1
1 to 6 days after last attack	8	–	1	–	–	–	1		–	–	–	2	1	1	1	1	–
1 week to 1 month after last attack	15	–	–	–	1	–	–		–	–	3	3	3	5	–	–	–
More than 1 month after last attack	14	–	–	–	–	–	–		–	2	1	2	4	1	3	1	–
Normal medical students	40	–	–	–	–	–	–		1	1	3	6	11	10	5	2	1

*The heavy vertical line represents the lower limit of normal.

DISCUSSION

The majority of patients examined did not have any attacks of asthma for a period of from one day to more than three months previous to the time the blood was examined. It is to be expected, therefore, that normal values should be found in these cases. It seems significant, however, that one-half of the cases examined during acute attacks of asthma should be found to have a definite hypomagnesemia. It appears even more significant that of the 16 individuals having a low serum magnesium, 13 should be suffering from an acute attack of asthma. The degree of hypomagnesemia, however, was not in proportion to the severity of the attacks. Patients S. R. and C. P. with relatively mild asthma were found to have magnesium values of 1.20 and 1.35 mg. per 100 c.c. serum, respectively. Patients E. S. and J. Q., on the other hand, suffered severe and persistent attacks, although the magnesium content of their blood was 1.53 and 1.60, respectively. Three individuals with a hypomagnesemia did not have any attacks for 2, 6, and 21 days, respectively.

That 50 per cent of the patients having acute attacks of asthma should have a low serum magnesium indicates that a hypomagnesemia and bronchial asthma may have some relationship. It appears quite plausible, at least in some cases, that a deficiency of this ion may upset the neuromuscular mechanism to such an extent as to make certain individuals more susceptible to bronchial spasms. It has been conclusively shown by Kruse and his collaborators[11, 12] and others[13-16] that such an ionic deficiency manifests itself chiefly by muscular spasms and convulsions. We must remain cognizant, however, of the possibility that the hypomagnesemia may be the result of the bronchial asthma.

Of equal importance is the fact that 50 per cent of the patients examined during attacks of asthma had normal serum magnesium. This means that a hypomagnesemia is by no means an essential etiologic factor. At present we may conclude that a disturbance in magnesium metabolism may be a contributing factor in a certain percentage of patients suffering from bronchial asthma. Further investigations in this direction should prove most interesting.

SUMMARY

1. The serum magnesium of 40 normal medical students varied between 1.74 and 3.10 mg. per 100 c.c., with a mean value of 2.33 mg.

2. The blood sera of 66 patients suffering with bronchial asthma were analyzed for their magnesium content with an analysis of the results.

3. The treatment of two patients with parenteral injections of magnesium sulfate is reported.

REFERENCES

1. Delbet, P., and Palios: Halogen Salts of Magnesium as Antianaphylactics, An Experimental Study, Bull. Acad. de méd., Paris 117: 96, 1937.
2. Lumiére, A., and Monchal, S.: Action of Intravenous Injections of Magnesium Thiosulfate on Cholesterol Content of Blood Studied in Relation to Its Use in Protection Against Anaphylactic Shock, Compt. rend. Soc. de biol. 124: 178, 1937.
3. Haury, V. G.: Unpublished observations.
4. Rosella, J. C., and Plá, J. C.: Magnesium Sulfate in Crisis of Asthma, Prensa méd. argent. 23: 1677, 1936.

5. Haury, V. G.: Modification of the Titan Yellow Method for the Determination of Small Amounts of Magnesium in Biological Fluids, J. Lab. & Clin. Med. **23**: 1079, 1938.
6. Becher, E.: Der Magnesium—Gehalt des Blutes unter normalen und pathologischen Verhältnissen, insbesondere bei Nierenkrankheiten, Klin. Wchnschr. **11**: 202, 1932.
7. Bomskov, C.: Determination of Magnesium in Blood With 8-hydroxyquinoline, J. Biol. Chem. **99**: 17, 1932.
8. Wacker, L., and Fahrig, C.: Über die mineralischen und lipoiden Bestandteile des Blutserums bei der essentiellen Hypertension im Vergleich zu den physiologischen Verhältnissen, Klin. Wchnschr. **11**: 762, 1932.
9. Walker, B. S., and Walker, E. W.: Normal Magnesium Metabolism and Its Significant Disturbances, J. Lab. & Clin. Med. **21**: 713, 1936.
10. Hirschfelder, A. D., and Haury, V. G.: Variations in Magnesium and Potassium Associated With Essential Epilepsy, Arch. Neurol. & Psychiat. **40**: 66, 1938.
11. Kruse, H. D., Orent, E. E., and McCollum, E. V.: Studies on Magnesium Deficiency in Animals, I. Symptomatology Resulting From Magnesium Deprivation, J. Biol. Chem. **96**: 519, 1932.
12. Orent, E. R., Kruse, H. D., and McCollum, E. V.: Studies on Magnesium Deficiency in Animals, II. Species Variation in Symptomatology of Magnesium Deprivation, Am. J. Physiol. **101**: 454, 1932.
13. Metzger, H. J.: A Case of Tetany With Hypomagnesemia in a Dairy Cow, Cornell Veterinarian **26**: 353, 1936.
14. Hopkirk, C. S. M.: Magnesium in "Grass Staggers," New Zealand Department of Agriculture, Annual Report, 1934-35.
15. Cunningham, I. J.: The Distribution of Magnesium in the Animal Organism and the Effect of Dietary Magnesium, New Zealand J. Sc. Tech. **18**: 419, 1936.
16. Seekles, L., and Sjellema, B.: Etiology of "Grass" Tetany, Arch. Tierheilk. **68**: 386, 1935.

SODIUM AND POTASSIUM STUDIES IN PERSONS WITH AND WITHOUT ARTERIAL HYPERTENSION*

Dudley W. Bennett, M.D., San Francisco, Calif.

THE relation of sodium and potassium to arterial blood pressure has not been studied extensively. It is, of course, well known that in adrenal cortical insufficiency (Addison's disease) hypotension and a low serum sodium and a high serum potassium occur; and conversely, in the hyperadrenal-cortical state the opposite is observed, namely, hypertension and a high serum sodium and low serum potassium.[1, 2] There is, however, no evidence to show that the altered sodium and potassium levels are directly related to the altered state of the blood pressure. Furthermore, McCance[3] has pointed out that the findings of a low serum sodium associated with a high blood pressure are quite common in interstitial nephritis.

The following study has been carried out to determine, if possible, whether the subject with arterial hypertensive disease might reveal some evidence of a disturbance in sodium and potassium metabolism when these two elements were drastically restricted in the diet.

A group of five male subjects was placed on a diet low in sodium and potassium content. Three of the subjects studied (J. L. B., aged 70 years; J. F. W., aged 58 years; and W. L. S., aged 28 years) had had definite elevation of systolic and diastolic blood pressures of from one to twelve years' duration, with

*From the Department of Medicine, University of California Medical School, San Francisco.
Received for publication, November 2, 1939.

or without evidence of renal disease but without evidence of abnormal retention of fluid. None of the subjects gave evidence of nitrogen retention in the blood.

The two persons in the control group (W. Y. B., aged 21 years and J. A. N., aged 24 years) had normal blood pressure readings and no evidence of fluid retention. W. Y. B. had an anxiety neurosis, and had had before the study was undertaken a systolic blood pressure reading as high as 150. His diastolic blood pressure was at all times within normal limits. Immediately before and during the study his systolic blood pressure was within normal limits.

All patients were confined to the hospital for observation for periods of from two to fifteen days before the study was begun. During the study their physical activities were restricted to such exercise as walking about the ward. At no time was abnormal sweating present. The environmental temperature was fairly constant, maintained at approximately 22° C. During a preliminary period of twenty-four hours, the fluid intake, urinary output, blood pressure, and body weight were determined. At the end of this period venous blood was obtained after the patient had been fasting for fourteen hours, for determination of the serum sodium, potassium, and protein; the plasma nonprotein nitrogen; and the blood sugar, chlorides, and carbon dioxide (see Table II and Chart 1).

The subjects were then given a special diet, which had been prepared under the supervision of Miss A. Huesman, of the Dietetic Department of the University of California Hospital.

SPECIAL MIXTURE*

Daily portion:

125 Gm. of dry uncooked white rice
125 Gm. of washed cottage cheese
10 Gm. of pure glucose

Method of preparation:

Wash rice thoroughly; drain. Mix rice with 1½ quarts of distilled water. Place in heavy kettle over direct heat. Boil for twenty minutes; drain in sieve. Mix rice that has been drained with 1 quart of distilled water and boil for ten minutes. Drain. Repeat last process and drain.

Purée commercially prepared cottage cheese through very fine strainer. Place in cheesecloth bag. Wash in 2 quarts tap water, kneading thoroughly; drain. Repeat process eight times. Repeat ninth time using distilled water. Drain overnight. Weigh 125 Gm. of washed cottage cheese.

Mix together cooked rice, washed cottage cheese, and glucose that has been dissolved in ½ cup distilled water. To this mixture add distilled water until the total weight of mixture equals 810 Gm.

Food value:

Protein	25 Gm.	
Fat	2 Gm.	
Carbohydrate	113 Gm.	
Total number of calories		610

A sample of each batch of food so prepared was analyzed for the sodium and potassium content (see Chart 1).

The fluid intake, exclusive of the diet described above, was limited to 1,000 c.c. of distilled water in twenty-four hours. It was found that this amount of

*A sufficient amount of food for each subject was made at one time for the duration of the test.

fluid satisfied the thirst requirement while taking the diet. Occasionally a patient would take even less than the amount permitted (see Table I).

All the determinations of sodium and potassium were made, using the method described by Butler and Tuthill[4] for the sodium, and that described by Shohl and Bennett[5] for the potassium with the modifications suggested by Strauss.[6] The urine sodium determinations were made as described by Butler and Tuthill[4] without modifications in technique. The other specimens for sodium and potassium determinations were first dried on a hot plate and then ashed in a muffle furnace at 600° C. for approximately eight hours. In collecting all the specimens, care was exerted to maintain a uniform procedure. No evidence of hemolysis was observed in any serum specimens.

TABLE I

EFFECT OF LOW SODIUM AND POTASSIUM INTAKE ON BLOOD PRESSURE, BODY WEIGHT, FLUID BALANCE, AND URINE

		NORMAL FASTING	24 HOURS LOW Na AND K REGIME FASTING	48 HOURS LOW Na AND K REGIME FASTING	72 HOURS LOW Na AND K REGIME FASTING	96 HOURS LOW Na AND K REGIME + 6 GM. KCl FASTING	120 HOURS LOW Na AND K REGIME + 6 GM. KCl FASTING
Blood pressure in mm. Hg	J. L. B.	200/95	180/80	180/80	180/85		
	W. Y. B.	140/80	120/75	130/70	125/70		
	J. F. W.	225/135	215/125	200/120	195/120	190/115	190/115
	W. L. S.*	125/95	145/95	135/90	145/100	120/85	125/90
	J. A. N.	130/75	125/70	120/70	130/70	120/65	125/70
Urine output c.c.	J. L. B.	1,355/1,000	501/400	369/400	264/700		
	W. Y. B.	1,848/2,150	1,368/1,000	720/850	1,297/1,000		
Fluid intake c.c. (Exclusive of diet)	J. F. W.	1,800/1,490	1,475/1,000	1,447/1,000	1,055/900	587/650	710/700
	W. L. S.	860/1,100	1,165/900	1,205/800	850/800	950/1,000	1,267/1,000
	J. A. N.	4,845/5,550	1,840/1,000	545/1,000	795/1,000	530/1,000	690/1,000
Body weight in kg.	J. L. B.	67	67	66.8	66.2		
	W. Y. B.	66.1	64.7	64.1	63.1		
	J. F. W.	58.4	58.2	57.4	56.4	56.4	56
	W. L. S.	60.2	59.4	59	58.4	57.4	56.8
	J. A. N.	69	67	66.3	66	65.3	65.3
Urine pH	J. L. B.	-/1.014	-/1.029	-/1.028	-/1.022		
Urine specific gravity	W. Y. B.	-/1.016	-/1.014	5.5/1.020	5.5/1.013		
	J. F. W.	5.25/1.012	5.25/1.012	5.2/1.011	5.25/1.014	5.5/1.016	5.5/1.019
	W. L. S.	6.5/1.023	5.25/1.014	6.2/1.011	5.25/1.014	5.25/1.014	5.3/1.017
	J. A. N.	6.75/1.008	7.0/1.013	5.25/1.028	5.25/1.023	5.57/1.024	5.75/1.024

*Blood pressure readings of W. L. S. were found to be as high as 200/135 several weeks before the study. He was confined to the hospital for fifteen days for observation before the study was begun.

All other chemical determinations on the blood serum and plasma were made by Mrs. T. Leake of the Central Laboratory of the University of California Hospital.

In each instance, the low sodium and potassium intake was continued for three days. During this time daily specimens of blood were obtained for determination of the sodium and potassium content of the serum when the individual had been fasting for fourteen hours. All urine excreted was collected in twenty-four-hour lots and was measured for amount, specific gravity, and pH (Table I). The twenty-four-hour output of sodium and potassium excreted in the urine was determined (Chart 2). The blood pressure and the body weight were likewise determined daily when the subject had been fasting (Table I). At

the end of the three-day period all the chemical determinations of the blood serum and plasma were repeated (Table II).

Three persons (J. F. W., W. L. S., and J. A. N.) were continued on the same diet and fluid intake but with the additional administration of 6 Gm. of potassium chloride in enteric coated tablets for two days longer.

Two other patients were studied in the same manner. The first was in the normal group and the second had a history of arterial hypertension of at least ten years' duration but had normal blood pressure readings during the study. The results obtained in these persons are not reported in this paper because of incomplete data. Such data as were obtained, however, in both persons were similar to those obtained in other normal persons.

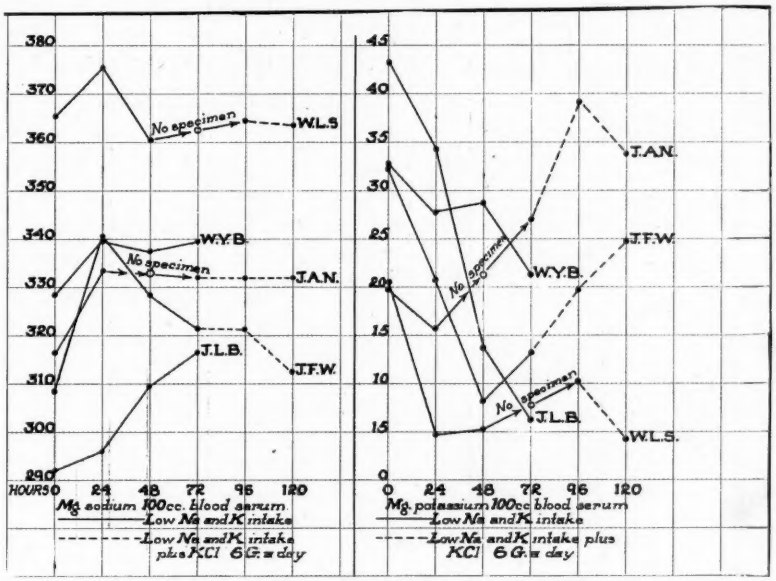


Chart 1.—Blood serum level of sodium and potassium, normal and low sodium and potassium intake.

		24 HOUR INTAKE	
		GM. Na	GM. K
W.L.S.	Essential hypertension (early) →	0.0149	0.0039
W.Y.B	Control →	0.0604	0.0210
J.F.W.	Essential hypertension (late) →	Trace	0.0454
J.L.B.	Essential hypertension (late) →	Trace	0.1831
J.A.N.	Control →	0.1220	0.04618

It was fully appreciated that the methods utilized in this study to restrict the intake of sodium and potassium were inadequate as far as normal diet requirements are concerned, and that the duration of the experiment was of a comparatively short period of time. However, it was felt that the reaction of the individual to salt deprivation might be tested in a more satisfactory manner by drastic restriction of both sodium and potassium for a shorter time than by permitting the person to have a less restricted intake of these two elements over a longer period of time. To prolong the drastic limitation of sodium and potassium intake would lead to a state of marked salt deficiency. It was felt that such a salt-deficient state would introduce faults in metabolism sufficient to render the interpretation of data obtained more difficult.

As mentioned above, no noticeable perspiration was present in any person during the experiment. No attempt, therefore, was made to measure or to

TABLE II

EFFECT OF LOW SODIUM AND POTASSIUM INTAKE ON BLOOD SERUM PROTEINS, BLOOD PLASMA CHLORIDES, NONPROTEIN NITROGEN, SUGAR, AND CARBON DIOXIDE

	J. L. B.		W. Y. B.		J. F. W.		W. L. S.		J. A. N.	
	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING
Blood serum proteins Total %	6.56	6.33	6.46	7.48	6.40	6.73	6.01	6.09	5.68	6.24
Albumin %	4.08	4.05	4.57	4.81	3.83	4.39	3.99	4.42	4.10	4.42
Globulin %	2.48	2.28	1.89	2.67	2.57	2.34	2.02	1.67	1.58	1.82
$\frac{\text{Albumin}}{\text{Globulin}}$	1.65	1.78	2.42	1.80	1.49	1.89	1.98	2.64	2.60	2.42
Plasma chloride in mg./100 c.c. (as NaCl)	592	567	599	591	561	602	574	582	595	556
Plasma nonprotein nitrogen in mg./100 c.c.	31.6	40.0	32.2	33.0	29.7	36.4	31.6	38.0	27.5	35.3
Plasma sugar %	0.093	0.115			0.084	0.081	0.098	0.078	0.104	0.084
Plasma carbon dioxide in c.c.					55.5	48.2	47.7	50.2	54.7	46.0

analyze the fluid lost in the perspiration or to provoke excessive loss of fluid by artificial means, as has been reported by McCance.[7]

Tables I and II give data other than the sodium and potassium determinations. Charts 1 and 2 show in graphic form the fasting daily sodium and potassium blood serum levels and the daily excretion of sodium and potassium in the urine; they also give notation of the sodium and potassium intake in twenty-four hours.

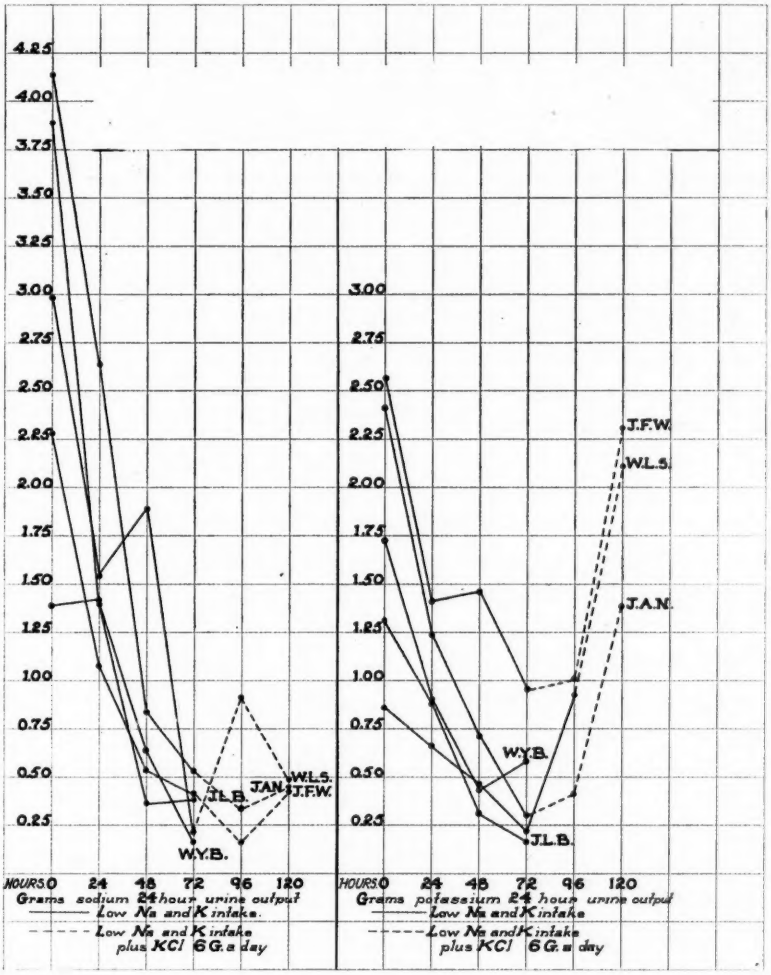


Chart 2.—Excretion of sodium and potassium in the urine, normal and low sodium and potassium intake.

DISCUSSION

Although the serum sodium level fluctuated in each individual when the sodium and potassium were restricted drastically, this change was of a minor degree in all five curves. When the two curves for the normal controls are compared with those for the patients with hypertension, no definite difference is seen between the levels of blood serum sodium in normal persons and the levels in persons with arterial hypertension.

The levels of serum potassium, however, fell following deprivation of sodium and potassium. It should be noted that in patients with definite arterial hypertension (J. L. B., J. F. W., and W. L. S.) a greater drop occurred than in two normal persons (W. Y. B. and J. A. N.). One normal individual (J. A. N.) at first had a moderate drop in the potassium level followed by a rise to above his normal level when sodium and potassium were restricted.

Chart 2 shows that the urinary output of both sodium and potassium decreased when these elements were restricted in the diet.

Two of the three patients (J. F. W. and W. L. S., both having arterial hypertension) on whom the experiment was continued for an additional two days with the addition of 6 Gm. of potassium chloride daily, failed to regain during this period their normal fasting levels of blood serum potassium. The change in the blood serum sodium in this group was of a minor degree. The addition of potassium chloride caused a definite increase in the excretion of potassium in the urine, with very little change in the amount of sodium excreted.

It was considered that the fall in blood pressure was probably due to several factors, namely, restriction of food and fluid intake as well as rest. Other data, such as fluid balance, body weight, and urinary pH shown in Table I, and serum protein, plasma chloride, sugar, and carbon dioxide combining power in Table II, showed only moderate variations and were considered to be of no great significance. It will be recalled that McCance[7] demonstrated very marked changes in such blood constituents as serum proteins, urea, chlorides, and sodium in his patients in whom he produced a sodium chloride deficiency. None of the persons reported in this paper complained of the symptoms reported by McCance, such as aberrations of flavor, cramps, weakness, and lassitude. It is quite safe to conclude from our data in Tables I and II that our patients were not in a salt-deficiency state.

A rise was noted in the nonprotein nitrogen level of the blood plasma in all persons following sodium and potassium restriction.

It is well known that potassium is held largely in the intracellular fluid and sodium is held in the extracellular fluid. It was found in this study that the potassium level of the blood serum falls when the intake of both sodium and potassium is restricted. The significance of the greater decrease in the serum level of potassium in the patients with arterial hypertension is not clearly understood at the present time.

SUMMARY AND CONCLUSIONS

In five male patients studied drastic restriction of sodium and potassium in the diet for a period of from three to five days produced no significant change in the blood serum level of sodium, but caused a drop in the blood serum level of potassium. This drop in the potassium level was of a greater magnitude in the three persons who had definite arterial hypertensive disease than in those who had normal blood pressure.

The urinary excretion of both sodium and potassium decreased with restriction of these two elements in the diet.

NOTE: Since the completion of the study here presented, my attention has been called to a paper by O. L. U. S. DeWesselow and W. A. R. Thomson entitled, "A Study of Some Serum Electrolytes in Hypertension," in the Quart. J. Med. **8**: 361, 1939. These authors pointed out that in their experiments the serum of patients suffering from essential and malignant hypertension tended to show a lower level of potassium than that of patients with a normal blood pressure on the same diet.

REFERENCES

1. McQuarrie, Irvine, Johnson, R. M., and Ziegler, M. R.: Plasma Electrolyte Disturbance in Patient With Hypercorticoadrenal Syndrome Contrasted With That Found in Addison's Disease, Endocrinology **21**: 762, 1937.
2. Anderson, Evelyn, Haymaker, Webb, and Joseph, Michael: Hormone and Electrolyte Studies of Patients With Hyperadrenocortical Syndrome (Cushing's Syndrome), Endocrinology **23**: 398, 1938.
3. McCance, R. A.: Medical Problems in Mineral Metabolism (The Goulstonian Lectures, 1936), Lancet **1**: 230, 704, 1936.
4. Butler, A. M., and Tuthill, Elizabeth: An Application of the Uranyl Zinc Acetate Method for Determination of Sodium in Biological Material, J. Biol. Chem. **93**: 171, 1931.
5. Shohl, Alfred T., and Bennett, Helen B.: A MicroMethod for the Determination of Potassium as Iodoplatinate, J. Biol. Chem. **78**: 643, 1928.
6. Strauss, Margaret B.: The Use of Thorium Nitrate in the Rapid Ashing of Serum and Urine. I. Adapted for Subsequent Potassium Determinations, J. Biol. Chem. **118**: 331, 1937.
7. McCance, R. A.: Experimental Sodium Chloride Deficiency in Man, Proc. Roy. Soc., London, s. B. **119**: 245, 1935.

THE EFFECT OF IRON ON PHOSPHORUS, CALCIUM, AND NITROGEN METABOLISM*

ADELAIDE P. BARER, PH.D., AND WILLIS M. FOWLER, M.D.
IOWA CITY, IOWA

THE administration of certain metals to experimental animals produces a low blood phosphorus and rickets. Both iron and aluminum,[1] when given in excess of the total phosphorus, were shown to precipitate the alimentary phosphorus as insoluble phosphates which were not absorbed and consequently caused a lowering of the blood phosphorus in guinea pigs. When ferric chloride[2] was added to a nonrachitogenic diet, it rendered this same diet rachitogenic for rats and a low serum phosphorus resulted. It was found that iron and ammonium citrates exerted the same effect and reduced iron acted similarly. The rachitogenic effect could be prevented by adding excess phosphorus to the diet. Deobald and Elvehjem[3] pointed out that the addition of a soluble iron salt to the diet in chicks produced severe rickets within five days and that the lowering of the blood phosphorus to this degree might affect other biologic processes as well as bone nutrition. The clinical implications from these animal experiments are obvious, and the possible harm resulting from the administration of large doses of iron during the period of growth and development of a child has been suggested. Brock and Diamond[2] state that large doses of iron continued over a

*From the Department of Internal Medicine, State University of Iowa, College of Medicine, Iowa City.
Received for publication, December 28, 1939.

TABLE I

CASE	DIAGNOSIS	AGE	SEX	BLOOD HEMOGLOBIN GM. %	MINERAL	CONTROL	PERIODS WITH IRON							
							I	II	III	IV	V	VI	VII	VIII
1	Anemia, hemorrhagic	45	F	7.80	Fe	+ 0.11	+11.62	+ 13.73						
					P	+ 0.24	+ 0.26	+ 0.28						
					Ca	- 0.06	- 0.18	- 0.19						
					N	+ 0.43	+ 0.84	+ 1.13						
2	Anemia, hemorrhagic	43	F	7.02	Fe	- 5.05	+14.06	+ 13.92						
					P	- 0.13	+ 0.37	+ 0.29						
					Ca	- 0.50	- 0.11	+ 0.18						
					N	- 0.21	+ 1.60	+ 1.92						
3	Anemia, hemorrhagic	42	F	7.60	Fe	- 2.66	+12.20	+ 5.45						
					P	± 0.00	+ 1.74	+ 1.04						
					Ca	- 0.72	- 1.18	- 1.93						
					N	+ 0.38	+ 3.96	+ 2.66						
4	Anemia, hemorrhagic	52	F	5.65	Fe	- 2.14	+ 5.87	+ 16.03	+ 16.49	+ 12.25				
					P	+ 0.22	+ 0.04	+ 0.20	+ 0.08	+ 0.06				
					Ca	- 0.31	- 0.87	- 0.01	- 0.64	- 0.75				
					N	+ 0.90	+ 2.27	+ 1.24	+ 1.27	+ 0.42				
5	Anemia, idiopathic	44	F	7.45	Fe	- 4.28	+53.25	+ 14.31	+ 65.40	+ 48.54				
					P	+ 0.03	+ 0.29	- 0.11	- 0.01	+ 0.23				
					Ca	- 0.80	+ 0.68	+ 0.36	+ 0.01	+ 0.77				
					N	- 0.24	+ 0.42	- 0.16	- 1.79	- 1.75				
6	Anemia, idiopathic	41	F	7.18	Fe	- 6.66	+55.13							
					P	+ 0.27	- 0.28							
					Ca	- 0.28	+ 0.09							
					N	- 1.34	- 0.87							

Iron expressed as milligrams.
Phosphorus, calcium, and nitrogen expressed as grams.

7	Anemia, hemorrhagic	21	F	7.89	Fe	- 9.53	+71.04	+ 61.21	+114.40					
					P	+ 0.17	- 0.03	+ 0.18	+ 0.10					
					Ca	+ 0.20	- 0.04	+ 0.03	+ 0.23					
					N	+ 4.76	+ 5.78	+ 5.18	+ 4.97					
8	Anemia, hemorrhagic	23	F	5.97	Fe	-12.70	+43.22							
					P	+ 0.25	+ 0.25							
					Ca	- 0.08	- 0.17							
					N	- 0.05	+ 2.04							
9	Anemia, hemorrhagic	33	F	3.61	Fe	-12.97	+43.14	+ 42.16						
					P	+ 0.22	- 0.16	- 0.16						
					Ca	+ 0.66	- 0.23	- 0.05						
					N	- 0.81	+ 4.64	+ 6.34						
10	Anemia, hemorrhagic	45	F	2.36	Fe	+ 3.01	+83.74	+ 27.59	+ 36.62	+ 43.45	+ 93.54	-103.92	+66.14	
					P	+ 0.25	+ 0.44	+ 0.15	- 0.26	+ 0.01	+ 0.03	+ 0.26	+ 0.24	
					Ca	+ 0.25	+ 0.31	+ 0.28	- 0.05	+ 0.38	- 0.11	+ 0.17	- 0.16	
					N	+ 1.11	+ 1.28	+ 1.93	+ 1.33	+ 1.69	+ 1.01	- 1.24	- 0.37	
11	Lead poisoning	55	F	7.89	Fe	+ 2.27	+153.22	+153.39	- 52.21	+ 19.56				
					P	+ 0.35	+ 0.29	+ 0.19	+ 0.25	+ 0.20				
					Ca	+ 0.78	+ 0.62	- 0.13	+ 0.64	+ 0.77				
					N	+ 2.51	+ 2.48	+ 1.61	+ 1.79	+ 0.70				
12	Anemia, idiopathic	34	F	5.60	Fe	- 1.37	+309.67	+289.38						
					P	+ 0.30	+ 0.48	- 0.11						
					Ca	+ 0.68	± 0.00	- 0.20						
					N	+ 0.32	+ 2.27	+ 1.49						
13	Anemia, hemorrhagic	52	F	5.65	Fe	- 2.14	+280.60	+ 83.97						
					P	+ 0.22	+ 0.27	- 0.31						
					Ca	- 0.05	- 0.09	- 0.94						
					N	+ 0.90	+ 1.52	+ 0.87						
14	Anemia, idiopathic	41	F	4.80	Fe	- 9.78	+282.61	+123.63	+283.53	+318.75	+330.42	+120.32	+97.06	-99.51
					P	- 0.18	- 0.04	+ 0.10	+ 0.09	- 0.21	- 0.13	- 0.22	+ 0.31	- 0.08
					Ca	+ 0.46	- 0.37	+ 0.23	+ 0.42	+ 0.63	+ 0.64	+ 0.10	+ 0.81	- 0.41
					N	- 2.09	- 0.12	- 0.05	+ 0.05	+ 0.48	+ 0.56	+ 0.02	- 1.21	- 2.48

TABLE I—CONT'D

CASE	DIAGNOSIS	AGE	SEX	BLOOD HEMOGLOBIN GM. %	MINERAL	CONTROL	PERIODS WITH IRON							
							I	II	III	IV	V	VI	VII	VIII
15	Anemia, hemorrhagic	42	F	7.60	Fe	- 2.66	+233.63	+254.01						
					P	± 0.00	+ 0.19	+ 0.04						
					Ca	- 0.72	- 0.47	- 0.67						
					N	+ 0.38	- 0.29	- 0.11						
16	Chronic arthritis	53	F	12.63	Fe	+ 3.45	+203.59	+200.27	+342.78					
					P	+ 0.24	+ 0.13	+ 0.33	+ 0.22					
					Ca	+ 0.11	+ 0.11	+ 0.18	+ 0.04					
					N	+ 1.06	+ 0.65	- 0.35	- 0.28					
17	Chronic arthritis	41	F	11.99	Fe	+ 2.64	+307.04	+333.77	+276.33					
					P	+ 0.33	+ 0.12	- 0.18	+ 0.37					
					Ca	+ 0.61	- 0.06	+ 0.62	+ 0.06					
					N	+ 0.71	- 0.03	+ 1.51	+ 1.18					
18	Chronic arthritis	51	F	12.41	Fe	- 1.63	+280.23	+288.62	+110.27	+220.65	+220.60			
					P	+ 0.13	+ 0.14	+ 0.09	- 0.02	+ 0.23	+ 0.25			
					Ca	+ 0.05	+ 0.20	+ 0.36	- 0.19	+ 0.54	- 0.01			
					N	+ 0.12	+ 0.57	- 0.68	- 0.90	- 0.49	- 0.50			
19	Anemia, idiopathic	43	F	4.08	Fe	- 2.90	+404.41	+304.47						
					P	- 0.07	- 0.32	- 0.30						
					Ca	+ 0.02	- 0.47	+ 0.25						
					N	- 0.69	- 1.06	- 0.64						

long period of time in childhood might have an effect similar to that observed in experimental animals, perhaps not by the actual production of rickets, but by evidences of malnutrition of the bones. Elvehjem[4] also warns of the danger of administering large amounts of iron and advises that the dose be kept at a minimum. With these points in mind balance studies were carried out on 19 adults to ascertain the effect of iron administration on phosphorus, calcium, and nitrogen retention.

METHOD

The method of study was the same as that described in the first of a series of reports on iron balances.[5] Sixteen patients with varying degrees of hypochromic anemia, either idiopathic in origin or secondary to hemorrhage, and 3 control patients without anemia were selected for the study. The control period was preceded by a three-day adjustment period on the balance diet before observations were begun. Each balance period was of six days' duration, and the results are reported as the average value per day. During the control period the iron intake was obtained from the diet alone. In subsequent periods iron and ammonium citrates were given in varying amounts. Nitrogen was determined by the Kjeldahl method, phosphorus by the method of Fiske and Subbarow,[6] and iron by the method of Reis and Chakmakjian.[7] Calcium determinations followed the method of McCrudden.[8]

The diets contained 1 Gm. of protein per kilogram of body weight, and the dietary iron intake varied from 9 to 12 mg. a day. The phosphorus and calcium intake, derived exclusively from the food, was approximately 1.5 and 1.3 Gm. a day, respectively.

RESULTS

A summary of the iron, phosphorus, calcium, and nitrogen balances for each patient is given by periods in Table I. The age of the patients, who were all females, varied from 21 to 53 years. An examination of the average daily balances during the control periods shows no correlation between the storage of the substances and the age of the patient. All patients except the controls (Cases 16, 17, and 18) had hypochromic anemia of varying degree. As has been pointed out previously, there is no correlation between the degree of anemia and the iron retention[9] either during the control period or with the administration of medicinal iron, and in this study the degree of anemia had no effect on the phosphorus, calcium, or nitrogen balances.

In Table II is given a summary of the balance studies showing the intake and balance for each of the four substances during both the control period and the period of iron administration. The results during the administration of iron are expressed as the average daily intake and balance for the period of observation, which varied from six to forty-eight days, rather than being given by periods. It will be seen that during the period of iron administration all patients retained this metal, the amount retained varying with the intake. Cases 1 to 4 received small amounts of iron intramuscularly, 1.5 grains of iron and ammonium citrates a day. Cases 5 to 11 received from 15 to 36 grains of iron and ammonium citrates orally a day, and Cases 12 to 19 received 45 grains of the same preparation by mouth.

TABLE II

CASE	IRON SUMMARY		PHOSPHORUS SUMMARY		CALCIUM SUMMARY		NITROGEN SUMMARY		DAYS	MEDICINAL IRON (GRAINS PER DAY)
	INGESTION	BALANCE	INGESTION	BALANCE	INGESTION	BALANCE	INGESTION	BALANCE		
1	11.89	+ 0.11	1.44	+0.24	1.25	-0.06	9.78	+0.43	6	0
	23.53	+ 12.68	1.45	+0.27	1.25	-0.18	9.79	+0.99	12	1.5
2	11.89	- 5.05	1.44	-0.13	1.25	-0.50	9.78	-0.21	6	0
	23.58	+ 13.99	1.50	+0.33	1.26	+0.04	10.41	+1.76	12	1.5
3	11.73	- 2.66	1.44	±0.00	1.26	-0.72	9.78	+0.38	12	0
	24.59	+ 8.87	1.45	+0.23	1.26	-0.26	9.84	+0.55	12	1.5
4	11.86	- 2.14	1.45	+0.21	1.26	-0.05	9.79	+0.90	6	0
	24.51	+ 12.66	1.45	+0.10	1.26	-0.57	9.82	+1.30	24	1.5
5	10.17	- 4.28	1.42	+0.03	1.27	-0.08	9.96	-0.24	6	0
	170.58	+ 46.72	1.42	+0.10	1.27	+0.45	9.97	-0.85	23	15
6	11.89	- 6.65	1.44	+0.27	1.25	-0.28	9.78	-1.34	6	0
	180.46	+ 55.13	1.43	-0.28	1.25	+0.09	9.85	-0.87	6	15
7	12.69	- 9.52	1.50	+0.17	1.28	+0.20	10.37	+4.76	6	0
	182.04	+ 82.22	1.55	+0.08	1.31	+0.07	11.04	+5.31	18	15
8	13.03	- 12.70	1.53	+0.25	1.29	-0.08	10.77	-0.05	6	0
	182.74	+ 43.22	1.62	+0.25	1.32	-0.17	11.85	+2.04	6	15
9	12.96	- 12.97	1.55	+0.22	1.31	+0.66	10.73	-0.81	6	0
	183.25	+ 42.65	1.72	-0.16	1.52	-0.12	12.49	+5.49	12	15
10	11.89	+ 3.01	1.44	+0.25	1.37	+0.24	9.78	+1.11	6	0
	267.31	+ 35.30	1.48	+0.12	1.37	+0.12	10.93	+0.80	42	36
11	11.49	+ 2.27	1.44	+0.35	1.34	+0.78	9.84	+2.51	6	0
	268.17	+ 68.48	1.49	+0.23	1.49	+0.48	11.01	+1.65	24	36
12	11.89	- 1.37	1.44	+0.30	1.34	+0.68	9.78	+0.32	6	0
	426.62	+299.52	1.44	+0.18	1.52	-0.10	10.93	+1.88	12	40
13	11.86	- 2.14	1.45	+0.21	1.26	-0.05	9.79	+0.90	6	0
	517.70	+182.29	1.45	-0.02	1.26	-0.52	10.11	+1.20	12	45
14	11.89	- 9.78	1.44	-0.18	1.25	+0.46	9.78	-2.09	6	0
	517.70	+182.11	1.44	-0.02	1.25	+0.26	10.08	-0.35	48	45
15	11.73	- 2.66	1.44	±0.00	1.26	-0.72	9.78	+0.38	12	0
	517.70	+243.82	1.44	+0.11	1.26	-0.57	10.14	-0.20	12	45
16	11.81	+ 3.45	1.45	+0.24	1.25	+0.11	9.87	+1.06	6	0
	517.47	+248.88	1.43	+0.23	1.24	+0.11	9.73	+0.01	18	45
17	11.78	+ 2.64	1.45	+0.33	1.27	+0.61	9.81	+0.71	6	0
	517.83	+305.71	1.46	+0.11	1.27	+0.21	10.16	+0.89	18	45
18	11.78	- 1.63	1.45	+0.13	1.27	+0.05	9.81	+0.12	6	0
	517.87	+224.08	1.46	+0.13	1.27	+0.18	10.17	-0.40	30	45
19	4.15	- 2.90	0.80	-0.07	0.71	+0.02	5.84	-0.69	12	0
	518.12	+354.44	1.04	-0.31	0.73	-0.11	9.54	-0.85	12	45

Iron expressed as milligrams.
Phosphorus, calcium and nitrogen expressed as grams.
Iron medication was iron and ammonium citrates.

The effects of iron administration on the retention of phosphorus, calcium, and nitrogen are best seen in Table III in which the three substances are considered separately.

Table III shows the change in the phosphorus balance in each patient by periods. In Column 1 is the state of balance during the control period ex-

TABLE III

CASE	PHOSPHORUS									CALCIUM									NITROGEN								
	CONTROL	PERIODS WITH IRON								CONTROL	PERIODS WITH IRON								CONTROL	PERIODS WITH IRON							
		I	II	III	IV	V	VI	VII	VIII		I	II	III	IV	V	VI	VII	VIII		I	II	III	IV	V	VI	VII	VIII
1	+	+	+							-	-	-							+	+	+						
2	-	+	+							-	+	+							-	+	+						
3	E	+	+							-	+	+							+	+	+						
4	+	-	-	-	-					-	-	+	-	-					+	+	+	+	-				
5	+	+	-	-	+					-	+	+	+	+					-	+	+	-	-				
6	+	-								-	+								-	+							
7	+	-	+	-						+	-	-	+						+	+	+	+					
8	+	+								-	-								-	+							
9	+	-	-							+	-	-							-	+	+						
10	+	+	-	-	-	-	+	-		+	+	+	-	+	-	-	-		+	+	+	+	+	-	-	-	
11	+	-	-	-	-					+	-	-	-	-					+	-	-	-	-				
12	+	+	-							+	0	-							+	+	+						
13	+	+	-							-	-	-							+	+	-						
14	-	+	+	+	-	+	-	+	+	+	-	-	-	+	+	-	+	-	-	+	+	+	+	+	+	+	-
15	E	+	+							-	+	+							+	-	-						
16	+	-	+	-						+	0	+	-						+	-	-	-					
17	+	-	-	+						+	-	+	-						+	-	+	+					
18	+	+	-	-	+	+				+	+	+	-	+	-				+	+	-	-	-	-			
19	-	-	-							+	-	+							-	-	+						

Control indicates state of balance for each substance: positive, negative, or in equilibrium. Subsequent periods with iron administration indicate increased or decreased retention as compared to control period regardless of actual state of balance.

pressed as positive, negative, or in equilibrium. The other columns show the increase or decrease in phosphorus storage as compared to the control period, a + sign indicating that more was retained than during the control period and a – sign indicating that less was retained even though the patient might still have been in an actual positive balance. It is seen that in the first period of iron administration 8 patients retained less phosphorus than in the control period and 11 retained more. In the second period 10 of the 17 patients retained less than during the control period and 7 retained more.

In the first 4 patients, who received small doses of iron intramuscularly, there are ten observation periods during iron administration and in four of these, all in one patient, there was a decrease in phosphorus retention. In the other six periods there was an increase of phosphorus retention over the control period. It will be noted in Case 4 that less phosphorus was retained in each of the four periods on iron therapy than during the control period, but the patient was still in a positive balance for each period (Table I) as well as in a positive balance for the entire observation period of twenty-four days (Table II). If the explanation for the diminished phosphorus retention in experimental animals is correct (a precipitation of insoluble phosphates in the alimentary tract), there would be no reason to expect that intramuscularly administered iron would affect the phosphorus balance and there is no evidence of such an effect in these cases.

In the second group of 7 patients (Cases 5 to 11) the iron was administered by mouth in moderate amounts, from 15 to 36 grains of iron and ammonium citrates a day. There are twenty-two balance periods during iron administration in this group, and in sixteen of these there was a decrease in phosphorus retention. In only six periods was more phosphorus retained than during the control period. This might seem to indicate that decreased phosphorus absorption and retention occurred, but when the results of the third group of patients who received larger amounts of iron are considered, the significance of the preceding group is lost. In the third group (Cases 12 to 19) 45 grains of iron and ammonium citrates were administered daily by mouth. In the twenty-seven periods of observation during iron administration, there were twelve in which there was a decreased retention of phosphorus and fifteen in which the retention was increased. Since this larger dose of iron did not decrease phosphorus retention, it seems doubtful whether the diminished retention in the preceding group of 7 patients was due to iron ingestion. There were a total of forty-nine observation periods during orally administered iron, and in twenty-one of these there was increased phosphorus retention and in twenty-eight a decreased retention.

In the entire group of 19 persons there were fifty-nine observation periods during iron administration, and in thirty-two of these periods less phosphorus was retained than during the control period, while in twenty-seven periods there was an increased phosphorus retention. There were 5 patients in actual negative phosphorus balance during iron administration, whereas there were 3 in negative balance in the control period. These results

are interpreted as indicating that there is no significant change in the retention of phosphorus in the adult as a result of the administration of iron either orally or intramuscularly. It is our impression that the tendency toward decreased phosphorus retention is too slight and too inconsistent to be of significance.

In Table III are presented in similar manner the effects of iron administration on the retention of calcium. In thirty-two of the fifty-nine balance periods there was a diminished calcium retention as compared to the control period; in twenty-five instances the retention was increased, and in two periods there was no change. These figures are almost identical with those obtained for phosphorus, but a comparison of the tables shows that in individual cases and periods there is no correlation between the phosphorus and calcium retention. In the twenty-five periods of increased calcium retention there was increased phosphorus retention in only fourteen and a diminished phosphorus retention in eleven. Of the thirty-three periods with diminished calcium retention only twenty-one also showed diminished phosphorus retention. In the ten observation periods during intramuscular iron therapy in the first 4 patients there are five periods with increased, and 5 with decreased, calcium retention. In the second group of 7 persons who received iron by mouth there are twenty-two observation periods. In nine of these the calcium retention was increased and in thirteen it was decreased. There were twenty-seven balance periods during iron therapy in the last 8 patients (Cases 12-19) who received larger amounts of iron. There were eleven periods with increased, and 14 with decreased, calcium retention, and two periods with no change from the control period. During the control period there were 9 persons with calcium balance and 9 persons who were in an actual negative calcium balance for the entire period of iron administration. These results indicate that iron administration does not significantly affect calcium retention.

Table III also contains data on nitrogen metabolism. It is seen that the administration of iron did not affect nitrogen retention, and that there is no correlation between the retention of nitrogen and the retention of either calcium or phosphorus.

SUMMARY

The phosphorus, calcium, and nitrogen balances of 19 adult patients were determined during the administration of medicinal iron. No consistent or significant changes in the metabolism of these substances could be detected during the administration of iron either intramuscularly or orally.

REFERENCES

1. Cox, G. J., Dodds, M. L., Wigman, H. B., and Murphy, F. J.: The Effects of High Doses of Aluminum and Iron on Phosphorus Metabolism, J. Biol. Chem. **92**: xi, 1931.
2. Brock, J. F., and Diamond, L. K.: Rickets in Rats by Iron Feeding, J. Pediat. **4**: 442, 1934.
3. Deobald, H. J., and Elvehjem, C. A.: The Effect of Feeding High Amounts of Soluble Iron and Aluminum Salts, Am. J. Physiol. **111**: 118, 1935.
4. Elvehjem, C. A., Siemers, A., and Mendenhall, D. R.: Effect of Iron and Copper Therapy on Hemoglobin Content of the Blood of Infants, Am. J. Dis. Child. **50**: 28, 1935.

5. Fowler, W. M., and Barer, A. P.: Retention and Utilization of Orally Administered Iron, Arch. Int. Med. **59**: 561, 1937.
6. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**: 375, 1925.
7. Reis, F., and Chakmakjian, H. H.: Colorimetric Method for Quantitative Determination of Iron in Blood in the Form of Dispersed Prussian Blue, J. Biol. Chem. **92**: 59, 1931.
8. McCrudden, F. H.: The Determination of Calcium in the Presence of Magnesium and Phosphates: The Determination of Calcium in Urine, J. Biol. Chem. **10**: 187, 1911.
9. Barer, A. P., and Fowler, W. M.: Influence of Gastric Acidity and Degree of Anemia on Iron Retention, Arch. Int. Med. **59**: 785, 1937.

ALTERATION OF GLUCOSE TOLERANCE IN PATIENTS WITH DISEASE OF PITUITARY, THYROID, AND ADRENAL GLANDS BY CHANGES OF DIET*

JAMES A. GREENE, M.D., AND L. W. SWANSON, M.D.
IOWA CITY, IOWA

THE numerous reports of disturbance of carbohydrate metabolism in patients with dysfunction of the pituitary, thyroid, and adrenal glands, together with the development of this disturbance in experimental animals with altered function of these endocrine glands, appear to have definitely established a relationship between the function of these glands and carbohydrate metabolism. It has been shown also that alteration of the metabolic mixture through diet will produce changes in carbohydrate metabolism.[1] It is important in the proper treatment of patients and of academic interest to ascertain whether or not the alteration of such metabolism is due directly to dysfunction of these endocrine glands or to changes in the metabolic mixture. The present study was instituted to obtain data regarding this question. These data, which we recognize as incomplete, is submitted in hopes of arousing interest in the subject.

METHOD

Glucose tolerance curves were obtained in patients with acromegalia, pituitary dwarfism, hyperthyroidism, myxedema, Addison's disease, and paroxysmal hypertension due to a pheochromocytoma. The tolerance was ascertained in the morning after the patient had fasted for from twelve to fourteen hours. Blood samples were obtained from the finger tip before, one-half, one, one and one-half, two, and two and one-half hours after administration of 50 Gm. of glucose in aqueous solution orally. The metabolic mixture was then altered by change of diet for from three to twenty-four days and the glucose tolerance test was repeated. In all instances the tolerance curves were obtained after two types of diets, and in most cases after three. One diet was of a high carbohydrate and low fat content, another was of low carbohydrate and high fat content, and a third, of both the carbohydrate and fat content the same proportion as that in an

*From the Department of Internal Medicine, State University of Iowa, College of Medicine, Iowa City.
Received for publication, January 30, 1940.

average hospital diet. The caloric value of the diets varied from patient to patient, but were approximately the same for all diets prescribed for any one patient.

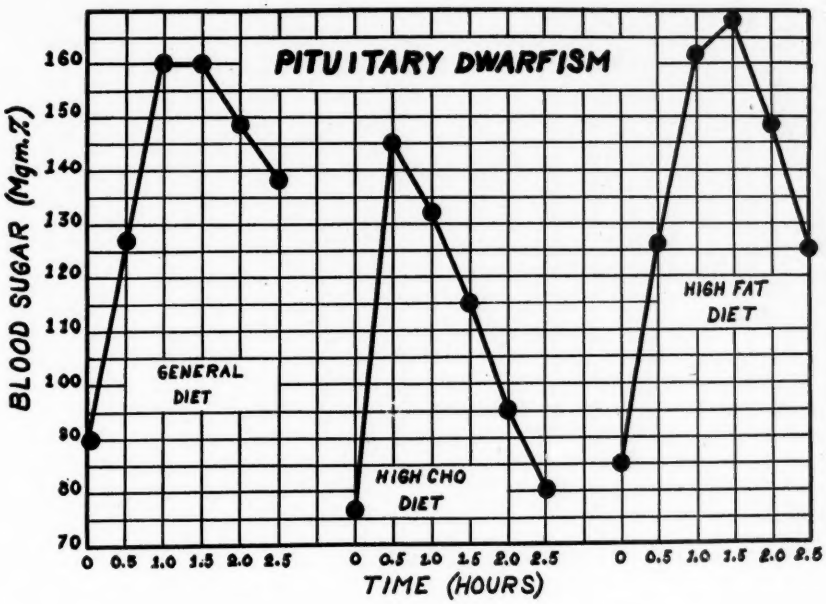


Fig. 1.

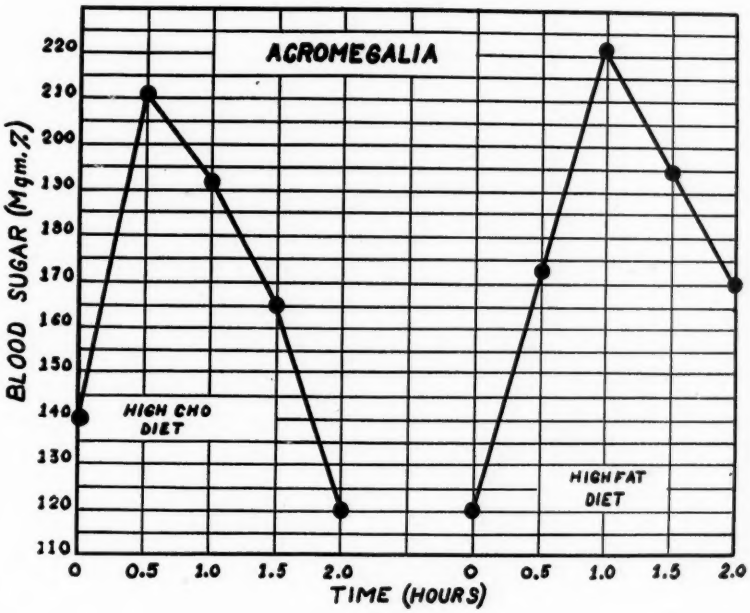


Fig. 2.

It was not deemed necessary to administer a given amount of glucose per kilogram of body weight for measure of the glucose tolerance because curves of one patient were not compared with those of another, but only with those of the same patient under different dietary conditions.

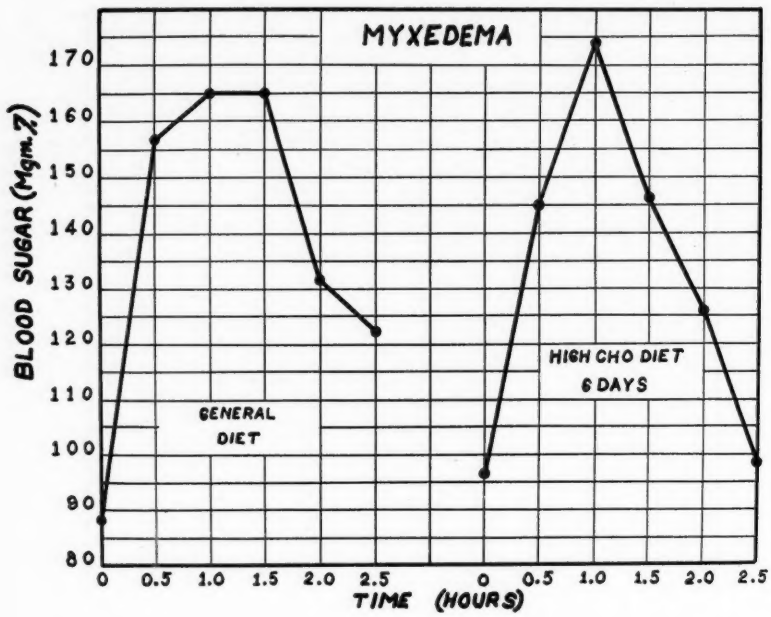

MYXEDEMA
BLOOD SUGAR (Mgm.%)
170
160
150
140
130
120
110
100
90
80
GENERAL DIET
HIGH CHO DIET 6 DAYS
0 0.5 1.0 1.5 2.0 2.5
0 0.5 1.0 1.5 2.0 2.5
TIME (HOURS)

Fig. 3.

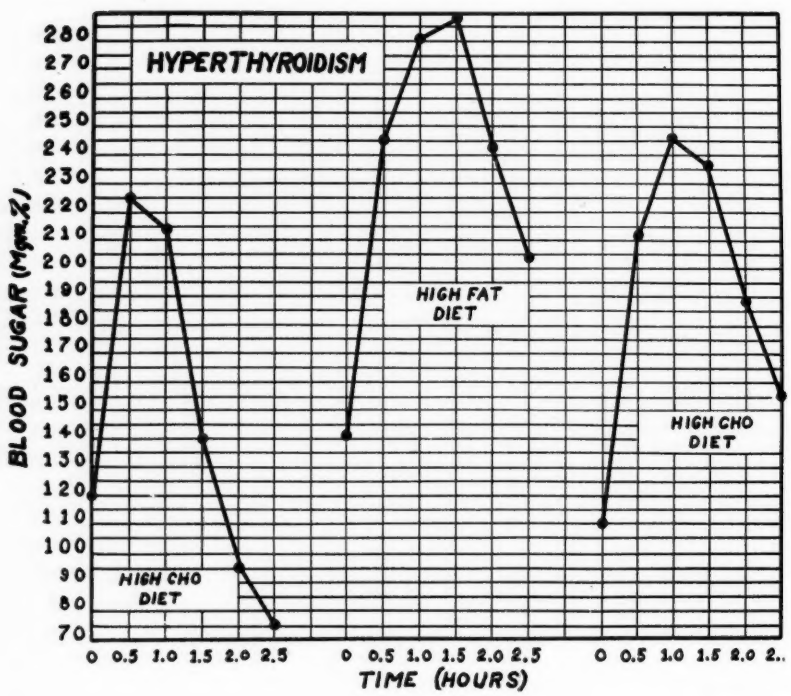

HYPERTHYROIDISM
BLOOD SUGAR (Mgm.%)
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
HIGH CHO DIET
HIGH FAT DIET
HIGH CHO DIET
0 0.5 1.0 1.5 2.0 2.5
0 0.5 1.0 1.5 2.0 2.5
0 0.5 1.0 1.5 2.0 2..
TIME (HOURS)

Fig. 4.

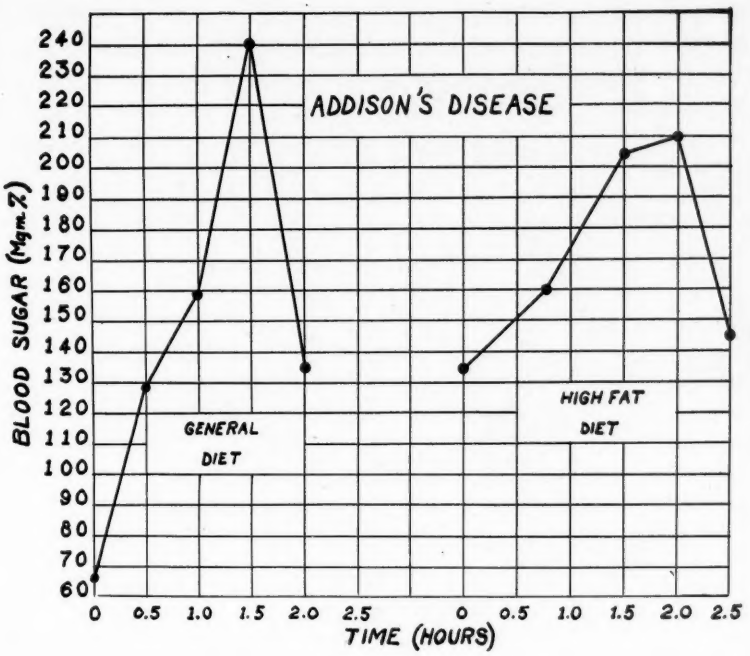

ADDISON'S DISEASE
BLOOD SUGAR (Mgm.%)
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
GENERAL DIET
HIGH FAT DIET
0 0.5 1.0 1.5 2.0 2.5
0 0.5 1.0 1.5 2.0 2.5
TIME (HOURS)

Fig. 5.

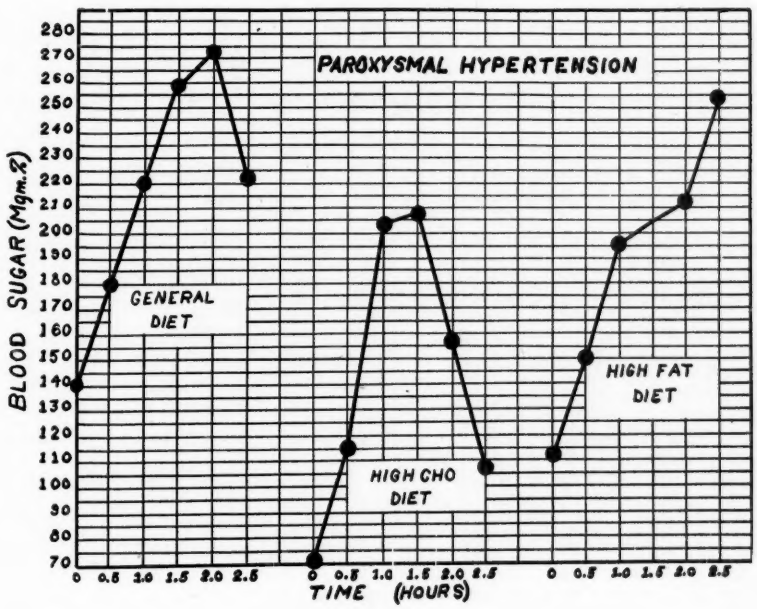

PAROXYSMAL HYPERTENSION
BLOOD SUGAR (Mgm.%)
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
GENERAL DIET
HIGH CHO DIET
HIGH FAT DIET
0 0.5 1.0 1.5 2.0 2.5
TIME (HOURS)

Fig. 6.

RESULTS

It is to be noted from Table I, which lists the number of patients with each disease and the effect of dietary alterations upon the glucose tolerance curve, that the tolerance was altered in all cases except in one of hyperthyroidism, and the results were questionable in one of Addison's disease. Representative alterations produced in the curve of each disease are shown in the accompanying graphs. Acetone bodies were not demonstrable in any of the seven patients in whom this point was ascertained during high fat, low carbohydrate diets.

TABLE I

NO. OF PATIENTS	DISEASE	EFFECT UPON GLUCOSE TOLERANCE	
		ALTERED	UNALTERED
2	Pituitary dwarfism	2	0
1	Acromegalia	1	0
2	Myxedema	2	0
8	Hyperthyroidism	7	1
1	Addison's disease	?	0
1	Pheochromocytoma	1	0
Total 15		13	1

COMMENT

It is recognized that there are too few cases of pituitary and adrenal dyscrasia in our study to permit any conclusions, but the results suggest that the disturbed carbohydrate metabolism may be due in part, if not entirely, to alteration of the metabolic mixture. If this is true, the disturbed carbohydrate metabolism can be corrected by proper dietary measures.

In regard to dysfunction of the thyroid gland, sufficient data are available to justify certain conclusions. There is usually a diminished glucose tolerance, but hypoglycemia may occur. Our results show that the diminished tolerance in hyperthyroidism is due to alteration of the metabolic mixture and that it can be remedied by a high carbohydrate and high caloric diet. The one patient who failed to show alteration of the tolerance curve had an initial normal curve and it was not altered by such a diet. The glucose tolerance can be diminished in these patients by a high fat and low carbohydrate diet. The one patient who failed to show this response developed hypoglycemia after two and one-half hours, and a high fat and low carbohydrate diet for eleven days thereafter did not alter the curve. A high carbohydrate and high caloric diet, on the other hand, was followed by a flattening of the curve and elimination of hypoglycemia. The explanation for this was not clear until we observed spontaneous hypoglycemia in patients due to a high fat diet.[2] It appears, therefore, that a high carbohydrate and high caloric diet is indicated in all cases of hyperthyroidism regardless of whether or not there is a diminished glucose tolerance or a hypoglycemia.

These observations show that the disturbed carbohydrate metabolism in hyperthyroidism is due to alteration of the metabolic mixture and is not due directly to dysfunction of the thyroid gland. The results show, in addition, that it is important to correct this altered glucose tolerance in such patients dur-

ing the preoperative treatment and that the maximum improvement should be one of the criteria which indicates whether or not the patient is prepared for thyroidectomy.

The patients with myxedema react to alterations of diet in the same manner as do normal persons, and this indicates that the diminished thyroid secretion is not a factor in the changes in carbohydrate metabolism in these cases.

SUMMARY

The glucose tolerance has been altered in patients with dysfunction of the pituitary, thyroid, and adrenal glands following alteration of the metabolic mixture by changing the carbohydrate and fat content of the diet. The tolerance was improved with a high carbohydrate diet and diminished with a high fat diet. It is suggested that in hyperthyroidism the maximum improvement in glucose tolerance be obtained before thyroidectomy is advised.

REFERENCES

1. Sweeney, J. S.: Dietary Factors That Influence the Dextrose Tolerance Test, Arch. Int. Med. 40: 818, 1927.
2. Swanson, L. W., and Greene, James A.: Further Observations on the Role of Diet in the Etiology and Treatment of Spontaneous Hypoglycemia, J. LAB. & CLIN. MED. To be published.

THE COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM*

KARL P. KLASSEN, M.D., RUTH L. BIERBAUM, S.B., AND GEORGE M. CURTIS, PH.D., M.D., COLUMBUS, OHIO

THE majority of patients with early, untreated exophthalmic goiter present an elevated blood iodine.[1] Veil and Sturm,[2] as early as 1925, demonstrated that there was an increase in the alcohol insoluble or "organic" iodine fraction in the blood of patients with hyperthyroidism. Later investigators have confirmed this observation.[3, 4] This increased "organic" iodine *presumably* represents a greater circulation of the high iodine containing thyroid hormone or of its higher metabolic products.

The level of the "inorganic" fraction of iodine in the blood shows considerable variation.[5] It is increased following the oral administration of inorganic iodine.[3] A portion of the "inorganic" iodine represents the iodine of nutrition, i.e., iodine derived from the food, water, and, to a limited extent, air; and which enters the blood stream principally from the gastrointestinal tract, but also from the lungs. Normally this fraction should be small considering the relatively low daily intake of iodine.

We have observed that in patients on a low iodine intake analyses of the blood cells reveal only a trace of iodine, this being consistently increased by the

*From the Department of Research Surgery, the Ohio State University, Columbus.
This investigation was aided by a grant from the Comly Fund for Research of the Ohio State University.
Received for publication, February 28, 1940.

oral administration of iodides. The present report deals with the results of a systematic investigation of the distribution of iodine in the serum and blood cells in patients without and with hyperthyroidsm before and following the oral administration of iodine.

METHODS

The investigation was carried out on four groups of patients. The *first* group was composed of ten individuals without thyroid disease and the *second* of ten patients with exophthalmic goiter. These patients were maintained on the usual hospital diet which has a relatively low iodine content.[6] They had not received iodine medication in any form for at least one month. The *third* group was made up of ten patients from the chest clinic, who had been lipiodolized for diagnostic purposes. No basal metabolic rate determinations were made on these patients; they showed no clinical evidence of thyroid disease. The *fourth* group was composed of ten patients with exophthalmic goiter who had received Lugol's solution or potassium iodide by mouth for from four to thirty days.

A sample of 70 c.c. of blood was drawn, with the patient in the fasting state. Twenty-five cubic centimeters of the blood were oxalated for the whole blood iodine determination. Forty cubic centimeters of blood were used to obtain from 15 to 25 c.c. of serum by clotting and centrifuging. Five cubic centimeters of blood were used for hematocrit determination. The analyses of the iodine in the whole blood and serum were made by the method of Matthews.[7]

TABLE I

IODINE CONTENT OF SERUM, BLOOD CELLS, AND WHOLE BLOOD

IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)*	IODINE IN BLOOD CELLS OF 100 C.C. OF WHOLE BLOOD (μg)	TOTAL IODINE IN SERUM PLUS CELLS OF 100 C.C. OF WHOLE BLOOD (μg)	IODINE CONTENT OF 100 C.C. OF WHOLE BLOOD BY ANALYSIS (μg)
4.2	0.2	4.4	4.2
5.0	0.4	5.4	5.2
35.0	2.3	37.3	38.1
924.0	203.0	1,127.0	1.058.0

*A microgram (μg) equals 0.001 mg.

The percentage of iodine in the serum was obtained by the use of the following formula, in which I_b and I_s represent the concentration of iodine in the whole blood and serum, respectively, while the cell volume of the blood is expressed by V:

$$\text{Per cent of iodine in serum} = \frac{I_s\ (100 - V)}{I_b}$$

As a check on the accuracy of the analytical methods employed, the actual iodine content of the blood cells was determined in four patients. Following the removal of the serum by centrifuging, the blood cells were washed five times with normal saline and then analyzed by the usual method. Twenty cubic centimeters of packed cells were used. The results of this procedure are shown in Table I. The results obtained by analyses of the whole blood as compared to those by adding the separately determined iodine content of serum and blood cells show only a small deviation. The present method of iodine analysis is accurate to within 5 per cent for the amount of iodine ordinarily found in blood.[7]

OBSERVATIONS

In the ten individuals without thyroid disease (Table II) the basal metabolic rate was within normal limits, averaging plus 5 per cent, with fluctuations of from minus 13 to plus 8. With a low iodine intake the whole blood iodine averaged 4.0 μg per cent, with variations of from 3.1 to 5.6 μg per cent. This is a normal blood iodine for this region by the present method of iodine analysis.[7] The serum iodine concentration showed a similar variation. It ranged from 5.9 to 10.2, averaging 7.1 μg per cent. One hundred cubic centimeters of blood contained from 3.1 to 5.0 μg of serum iodine, with a mean value of 3.8 μg. The blood cells of 100 c.c. of whole blood contained only 0.2 μg of iodine, taking the average of all ten determinations. The serum iodine thus constituted, on the average, 95 per cent of the total iodine of the blood.

TABLE II

COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM

Individuals Without Thyroid Disease

No Iodine Medication

CASE NO.	DIAGNOSIS	B.M.R. %	BLOOD IODINE μg %	SERUM IODINE μg %	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	Obesity	+ 4	3.1	6.9	53	3.2	103
2	Normal male	+ 6	3.2	5.9	48	3.1	97
3	Psychoneurosis	- 3	3.3	5.9	46	3.2	96
4	Normal male	- 8	3.7	6.8	50	3.4	92
5	Ulcer of leg	+ 8	3.9	6.6	47	3.5	91
6	Normal male	- 1	4.0	7.0	47	3.7	93
7	Normal male	+ 5	4.0	7.4	43	4.2	105
8	Normal female	+ 6	4.2	7.2	43	4.1	98
9	Arthritis	+12	5.1	7.1	37	4.5	88
10	Normal female	-13	5.6	10.2	51	5.0	90
Averages		+ 5	4.0	7.1	46	3.8	95

The basal metabolic rate was elevated in the ten patients with exophthalmic goiter (Table III). The lowest basal rate was plus 39, the highest was plus 85 per cent, averaging plus 60 per cent. The whole blood iodine was increased in all ten patients, ranging from 5.5 to 15.7 μg per cent. The average of 10.0 μg per cent was two and a half times greater than the normal blood iodine. The serum iodine showed a similar elevation, fluctuating from 10.4 to 25.0 μg per cent in these patients with exophthalmic goiter. The average was 18.0 μg per cent as compared with 7.1 in the patients without thyroid disease. The serum of 100 c.c. of whole blood contained from 5.3 to 14.7 μg of iodine, averaging 9.6 μg. The blood cells of the same amount of whole blood contained only 0.4 μg. In these patients 95 per cent of the whole blood iodine was in the serum.

In the ten patients without thyroid disease, but who had been lipiodolized, the whole blood and serum iodine reached an unusually high level (Table IV). This increased blood iodine was due to the high absorption of the iodine of the lipiodol from the gastrointestinal tract. Fluoroscopy following lipiodolization revealed the presence of lipiodol in the stomach in all cases. The absorption from the bronchial tree is low, as has been shown in a previous report.[8] The whole blood iodine ranged from 18.5 to 3153.0 μg per cent, with a serum iodine concentration of from 25.7 to 4232.0 μg per cent. The serum of 100 c.c. of whole

TABLE III

COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM

Patients With Exophthalmic Goiter

No Iodine Medication

CASE NO.	DURATION OF SYMPTOMS	B.M.R. %	WHOLE BLOOD IODINE μg %	SERUM IODINE μg %	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	2 years	+40	5.5	10.4	49	5.3	96
2	1 year	+56	6.1	10.1	42	5.9	97
3	6 months	+46	8.9	17.0	45	9.3	104
4	1 year	+63	8.9	15.4	41	9.0	100
5	1 year	+84	9.6	16.3	44	9.1	95
6	6 months	+39	10.8	17.6	44	9.8	90
7	6 months	+65	11.2	22.0	51	10.8	97
8	3 years	+56	11.4	20.7	53	9.8	86
9	1 year	+85	12.3	25.8	51	12.6	102
10	6 months	+64	15.7	25.0	43	14.7	90
Averages		+60	10.0	18.0	46	9.6	95

TABLE IV

COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM

Patients Without Thyroid Disease

Iodine Medication*

CASE NO.	DIAGNOSIS	WHOLE BLOOD IODINE μg %	SERUM IODINE μg %	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	Fracture of tibia	18.5	25.7	56	11.3	61
2	Chronic empyema	45.7	66.0	52	31.7	70
3	Pneumocyst of lung	72.0	124.8	53	58.6	81
4	Pulmonary moniliasis	474.0	521.0	47	276.0	60
5	Arteriosclerosis	1,058.0	1,777.0	48	924.0	88
6	Bronchiectasis	1,666.0	2,476.0	48	1,288.0	77
7	Bronchiectasis	1,672.0	2,518.0	49	1,284.0	77
8	Bronchiectasis	2,539.0	3,407.0	41	2,010.0	79
9	Obesity	2,467.0	3,724.0	45	2,048.0	82
10	Pulmonary tuberculosis	3,153.0	4,232.0	42	2,455.0	78
Averages				48		75

*Blood drawn from twelve to twenty-four hours following lipiodolization.

TABLE V

COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM

Patients With Exophthalmic Goiter

Iodine Medication

CASE NO.	IODINE MEDICATION	B.M.R. %	WHOLE BLOOD IODINE μg %	SERUM IODINE μg %	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	Lugol's 1 week	+60	15.2	20.2	40	12.2	80
2	Lugol's 4 days	+36	16.5	21.2	40	12.7	77
3	Lugol's 4 days	+49	17.3	22.0	36	14.1	80
4	100 mg. KI 5 days	+39	40.3	50.8	50	25.4	63
5	100 mg. KI 5 days	+ 8	41.5	53.7	46	29.0	70
6	100 mg. KI 9 days	+22	44.0	62.0	47	33.0	75
7	100 mg. KI 5 days	+38	63.5	74.5	43	42.5	67
8	100 mg. KI 5 days	+22	69.4	95.2	53	50.5	73
9	Lugol's 5 days	+38	106.0	162.0	50	81.0	76
10	100 mg. KI 30 days	+40	107.0	130.0	51	63.7	60
Averages		+35			46		72

blood contained from 11.3 to 2455.0 μg of iodine. There was an absolute, as well as a relative, increase in the corpuscular iodine, the cells of 100 c.c. of blood containing from 7.2 to 698.0 μg of iodine, or from 12 to 39 per cent of the total blood iodine.

Although the total iodine concentration in the patients with exophthalmic goiter who had received iodine in the form of Lugol's solution or potassium iodide was not as high as in these lipiodolized, the percentage of iodine in the serum to the total blood iodine was approximately the same (Table V). The basal metabolic rates were lower than in the patients with exophthalmic goiter not on iodine medication, and averaged plus 35 per cent, ranging from plus 8 to plus 60. The lowest whole blood iodine was 15.2 and the highest was 107.0 μg per cent. The iodine of the serum showed a corresponding increase, ranging from 20.2 to 130.0 μg per cent. The serum of 100 c.c. of whole blood contained from 12.2 to 63.7 μg, or on an average, only 72 per cent of the total blood iodine. The blood cells of the same amount of whole blood contained from 3.0 to 43.3 μg of iodine, as compared with 0.4 μg in the patients with exophthalmic goiter but not on iodine medication.

DISCUSSION

The increased whole blood iodine in the patients with untreated exophthalmic goiter as compared with the normal blood iodine of the individuals without thyroid disease was due to the higher iodine concentration of the serum, and not to an increased iodine content of the cells. With a low iodine intake the corpuscular iodine was approximately the same in both conditions. It constituted, on an average, only 5 per cent of the total blood iodine. The iodine concentration of the cells was thus similar to that of the cerebrospinal fluid of patients both with and without hyperthyroidism, as previously reported.[9] The average cerebrospinal fluid iodine concentration was found to be 0.5 μg per cent, as compared with the average corpuscular iodine concentration of 0.6 μg per cent in the twenty patients on a low iodine intake.

The corpuscular iodine would appear to represent the diffusible iodine fraction of the blood, and, we may assume, also a part of the nutritional iodine of the blood. An increased intake of iodine is followed by an increase in the diffusible iodine fraction of the blood, as evident by the higher corpuscular iodine concentration. However, this corpuscular iodine represents only a part of the nutritional iodine, as there is also a concomitant rise in the serum iodine content. A portion of the ingested iodine is diffusible, while the remaining part is retained in the serum.

The actual amount of iodine ingested by these patients was not determined. No attempt has been made to demonstrate a quantitative relationship between the iodine intake and the nutritional iodine concentration of the serum and cells.

The determination of the whole blood and serum iodine is of some practical value in the differential diagnosis of hyperthyroidism in that it makes possible the demonstration of an increased amount of diffusible iodine in the blood. An elevated whole blood iodine may be due to the increased serum iodine of hyperthyroidism, but may also be caused by an elevated nutritional iodine, brought about by the ingestion of high iodine content foods or the administration of

iodine. The finding of a high corpuscular iodine concentration indicates that the patient under consideration has received iodine medication, and that this is responsible for the elevated whole blood iodine.

SUMMARY

In ten individuals without thyroid disease and maintained on a low iodine diet; with normal metabolic rates and normal whole blood iodine averaging 4.0 μg per cent, the average serum iodine was 7.1 μg per cent. The serum iodine constituted, on an average, 95 per cent of the total blood iodine.

In ten patients with exophthalmic goiter, with an average basal metabolic rate of plus 60 per cent, and maintained on a low iodine intake, the average blood iodine was 10.0 μg per cent, the average serum iodine was 18.0 μg per cent. On an average, 95 per cent of the iodine of the blood was in the serum.

Following lipiodolization of ten patients without thyroid disease, the whole blood iodine ranged from 18.5 to 3153.0 μg per cent, with the serum iodine fluctuating from 25.7 to 4,232.0. From 61 to 88 per cent, or on an average 75 per cent, of the total iodine of the blood was in the serum.

In ten patients with exophthalmic goiter, with an average basal metabolic rate of plus 35 per cent, while receiving Lugol's solution or potassium iodide by mouth, the blood iodine concentration fluctuated from 15.2 to 107.0 μg per cent, with the serum iodine concentration varying from 20.2 to 130.0. From 60 to 80 per cent, or at an average 72 per cent, of the iodine of the blood was in the serum.

CONCLUSIONS

In individuals without thyroid disease and with a normal blood iodine, and also in those patients with exophthalmic goiter with an elevated blood iodine while on a low iodine intake, an average of 95 per cent of the total iodine of the blood is in the serum.

The elevated blood iodine of patients with exophthalmic goiter is due to an increased amount of serum iodine.

Following the oral administration of varying amounts of iodine or lipiodolization, there is an increased corpuscular iodine concentration, with a relative fall in the serum iodine to an average of 74 per cent of the total iodine of the blood.

REFERENCES

1. Curtis, G. M., and Puppel, I. D.: The Iodine Metabolism in Exophthalmic Goiter, Ann. Surg. **108:** 574, 1938.
2. Veil, W. H., and Sturm, A.: Beiträge zur Kenntnis des Jodstoffwechsels, Deutsches Arch. f. klin. Med. **147:** 166, 1925.
3. Holst, J., Lunde, G., Closs, K., and Pedersen, O. C.: Ueber den inneren Jodstoffwechsel bei primären Thyreotoxikosen (Primär-Basedow), Klin. Wchnschr. **7:** 2287, 1928.
4. Gutzeit, K., and Parade, G. W.: Blutjodstudien; die Blutjodfraktionen, Ztschr. f. klin. Med. **133:** 513, 1938.
5. Elmer, A. W.: Iodine Metabolism and Thyroid Function, London, 1938, Oxford University Press, pp. 100; 377-378.
6. Cole, V. V., Curtis, G. M., and Bone, M. L.: The Iodine Content of Hospital Foods, J. Am. Dietet. A. **10:** 200, 1934.
7. Matthews, N. L., Curtis, G. M., and Brode, W. R.: Determination of Iodine in Biological Materials, Indust. & Engin. Chem., Anal. Ed. **10:** 612, 1938.
8. Cole, V. V., Dunn, R. H., and Curtis, G. M.: The Intrapulmonic Absorption of Iodine, J. Pharmacol. & Exper. Therap. **53:** 327, 1935.
9. Klassen, K. P., Bierbaum, R. L., and Curtis, G. M.: The Comparative Iodine Content of Blood and Cerebrospinal Fluid, J. Lab. & Clin. Med. **25:** 383, 1940.

COMPARATIVE STUDIES ON THE ABSORPTION OF SULFANILAMIDE*

WALTER J. SIEBERT, M.D., AND FREDERICK LOOSE
ST. LOUIS, MO.

RECENT studies[2] of a solution of sulfanilamide† demonstrated that in the guinea pig and rabbit higher blood-free sulfanilamide concentrations are obtained with the solution than when sulfanilamide is given in equivalent dosage in other forms. Furthermore, the sulfanilamide appears in the blood more quickly when given as the solution, and its rate of elimination is not accelerated. The solution employed was essentially a mixture containing in each fluidounce approximately 30 grains of sulfanilamide in glucose, glycerin, and sodium lactate.

The question has arisen as to whether the sulfanilamide in the solution would be absorbed as rapidly as an equivalent amount of drug in the dry form, because of a possible linkage with the glucose of the solution. It has been postulated, also, that in persons with partial or complete achlorhydria and rapid gastric emptying time the sulfanilamide, if bound to glucose in the solution, might not be absorbed as quickly or as completely as when administered in dry form.

To answer these questions studies were made, employing the solution of sulfanilamide in glucose, glycerin, and sodium lactate, and for comparison a brand of sulfanilamide tablets accepted by the Council on Pharmacy and Chemistry of the American Medical Association. The subjects were five males and one female between the ages of 21 and 49 in apparent good health, and one male, 49 years old, and one female, 51 years old, both of whom were afflicted with pernicious anemia.

Vitamin C determinations were made whenever possible for the purpose of trying to establish some correlation between vitamin C gain or loss and the sulfanilamide concentration. While these cases are not sufficient to draw any conclusions, there is enough evidence of such correlation to warrant further investigation.

Quantitative determination of blood sulfanilamide was made according to the method of Bratton and Marshall.[1] Since a comparison was being drawn between free sulfanilamide and a solution which might possibly contain the drug in a conjugated form, all values given are in terms of free sulfanilamide. Total sulfanilamide, that is, free plus conjugated, was not determined.

*From the Department of Pathology, De Paul Hospital, St. Louis.
Received for publication, May 24, 1940.

†The solution of sulfanilamide known as "solution of sulfanilamide in glucose, glycerin, and sodium lactate," was furnished through the courtesy of Donley-Evans & Co., St. Louis.

PROCEDURE AND RESULTS

The first patient was a white male, aged 26 years, weighing 190 pounds. At the beginning of the study his red blood cell count was 4,140,000; hemoglobin, 13.8 Gm. (Newcomer method); white blood cell count, 9,600; nonprotein nitrogen, 40 mg. per cent; blood sugar, 92 mg. per cent; blood vitamin C, 0.15 mg. per cent. Fifteen cubic centimeters of the solution of sulfanilamide (equivalent to 15 grains of sulfanilamide) was administered every four hours. Sulfanilamide determinations were carried out on the blood at approximately 25° C. one-half hour before the drug was given and at four-hour intervals thereafter. The results are shown in Table I.

TABLE I

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 63 volumes per cent
12 noon	15 gr.	0.7	
4 P.M.	15 gr.	1.1	
8 P.M.	15 gr.	1.4	
12 midnight	15 gr.	1.8	
4 A.M.	15 gr.	2.3	
8 A.M.	15 gr.	2.7	
12 noon	Discontinued	2.9	Carbon dioxide combining power 57 volumes per cent
4 P.M.		2.1	
8 P.M.		1.6	
12 midnight		1.1	
4 A.M.		0.8	
8 A.M.		0.6	
12 noon		Not done	Carbon dioxide combining power 61 volumes per cent

A period of one week was allowed to elapse between the conclusion of the observations in Table I and the beginning of the following study. Fifteen grains of sulfanilamide tablets were administered every four hours as previously. The results are given in Table II.

TABLE II

SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 67 volumes per cent
12 noon	15 gr.	0.5	
4 P.M.	15 gr.	0.9	
8 P.M.	15 gr.	1.2	
12 midnight	15 gr.	1.5	
4 A.M.	15 gr.	1.9	
8 A.M.	15 gr.	2.2	
12 noon	Discontinued	2.4	Carbon dioxide combining power 54 volumes per cent
4 P.M.		2.0	
8 P.M.		1.6	
12 midnight		1.4	
4 A.M.		1.1	
8 A.M.		0.7	
12 noon		0.3	Carbon dioxide combining power 58 volumes per cent

The nonprotein nitrogen immediately following this study was 40 mg. per cent; the blood sugar, 87 mg. per cent. The blood picture showed no changes.

In this series of observations the same patient was used as in the first study, but after a period of two weeks. At this time, the nonprotein nitrogen was 42 mg. per cent, and the blood sugar was 87 mg. per cent. Blood vitamin C was 0.13 mg. per cent immediately before and 0.04 mg. per cent immediately following the experiment. Fifteen cubic centimeters of the solution of sulfanilamide was administered every four hours; sodium bicarbonate (approximately 20 to 30 gr. every two hours) was given throughout the test period in sufficient quantity to keep the urine alkaline during that time. Sulfanilamide determinations were made on the blood one-half hour before each dose of the drug was administered. The results are given in Table III.

TABLE III

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE, AND SODIUM BICARBONATE

TIME	DOSAGE	MILLIGRAMS PER CENT	URINE	
8 A.M.	15 gr.	0.0	Alkaline	Carbon dioxide combining power 68 volumes per cent
12 noon	15 gr.	0.4	Alkaline	
4 P.M.	15 gr.	0.7	Alkaline	
8 P.M.	15 gr.	1.0	Alkaline	
12 midnight	15 gr.	1.7	Alkaline	Carbon dioxide combining power 84 volumes per cent
4 A.M.	15 gr.	2.1	Alkaline	
8 A.M.	15 gr.	2.4	Alkaline	
12 noon	Discontinued	2.9	Alkaline	Carbon dioxide combining power 86 volumes per cent

The second patient was a white male, aged 30 years, weighing 194 pounds. At the start of the experimental period his red blood cell count was 4,870,000; hemoglobin, 15.1 Gm. (Newcomer method); white blood cell count, 8,400; nonprotein nitrogen, 24 mg. per cent; blood sugar, 81 mg. per cent. A blood vitamin C determination was not done at this time, but several days later the blood vitamin C was 1.73 mg. per cent. Fifteen cubic centimeters of the solution of sulfanilamide (equivalent to 15 grains of drug) was administered every four hours. Blood sulfanilamide determinations were made at approximately 25° C. one-half hour before each dose of the solution was administered. The results obtained are shown in Table IV.

TABLE IV

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 71 volumes per cent
12 noon	15 gr.	0.4	
4 P.M.	15 gr.	0.8	
8 P.M.	15 gr.	1.3	
12 midnight	15 gr.	1.7	
4 A.M.	15 gr.	2.1	
8 A.M.	15 gr.	2.6	
12 noon	Discontinued	2.8	Carbon dioxide combining power 69 volumes per cent
4 P.M.		2.4	
8 P.M.		2.0	
12 midnight		1.3	
4 A.M.		0.8	
8 A.M.		0.2	
12 noon		0.2	Carbon dioxide combining power 67 volumes per cent

A period of one week elapsed between the close of this and the following series of observations. The nonprotein nitrogen immediately preceding this series was 26 mg. per cent, and immediately following, 28 mg. per cent. Blood sugar determinations were not

made at this time. There were no significant hematologic changes. A dose of 15 grains of the brand of sulfanilamide tablets (5 gr.) previously employed was administered every four hours as shown in Table V.

TABLE V

SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 68 volumes per cent
12 noon	15 gr.	0.3	
4 P.M.	15 gr.	0.7	
8 P.M.	15 gr.	1.1	
12 midnight	15 gr.	1.2	
4 A.M.	15 gr.	1.5	
8 A.M.	15 gr.	1.8	
12 noon	Discontinued	2.0	Carbon dioxide combining power 61 volumes per cent
4 P.M.		2.1	
8 P.M.		1.4	
12 midnight		1.1	
4 A.M.		0.3	
8 A.M.		0.1	
12 noon		Not done	Carbon dioxide combining power 58 volumes per cent

The third patient was a white male, 28 years old, weighing 181 pounds. His red blood cell count immediately preceding the observations was 4,970,000, with a Newcomer hemoglobin of 15.6 Gm.; the white blood cell count, 6,500; the nonprotein nitrogen, 29 mg. per cent; the blood sugar, 72 mg. per cent.

Neither the white blood cell count nor the hemogram showed any appreciable change subsequently. The blood vitamin C three days before the study was started was 1.47 mg. per cent as determined with a photoelectric adaptation of Ingall's method for blood vitamin C. Fifteen cubic centimeters of the solution of sulfanilamide (equivalent to 15 grains of drug) was administered every four hours. Sulfanilamide determinations at 25° C. were made on the blood one-half hour before each dose of the drug was administered. Concomitantly, carbon dioxide combining power of the blood was determined as shown in Table VI.

TABLE VI

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 76 volumes per cent
12 noon	15 gr.	0.2	
4 P.M.	15 gr.	0.7	
8 P.M.	15 gr.	1.3	
12 midnight	15 gr.	1.5	
4 A.M.	15 gr.	1.9	
8 A.M.	15 gr.	2.1	
12 noon	Discontinued	2.3	Carbon dioxide combining power 72 volumes per cent
4 P.M.		2.0	
8 P.M.		1.6	
12 midnight		1.1	
4 A.M.		0.4	
8 A.M.		0.2	
12 noon		Not done	Carbon dioxide combining power 67 volumes per cent Vitamin C determination 0.96 milligrams per cent

A period of one week was allowed to elapse between the foregoing and the following series. The nonprotein nitrogen immediately preceding was 27 mg. per cent, and immediately following, 28 mg. per cent. Neither blood sugar nor vitamin C determinations were done at this time. The hemogram and red and white blood cell counts were essentially the same as at the beginning of the series. Fifteen grains of the previously employed brand of sulfanilamide tablets were administered every four hours. As before, sulfanilamide determinations were made on the blood one-half hour before each dose of the drug was administered. Results are shown in Table VII.

TABLE VII

SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 73 volumes per cent
12 noon	15 gr.	0.2	
4 P.M.	15 gr.	0.5	
8 P.M.	15 gr.	0.9	
12 midnight	15 gr.	1.3	
4 A.M.	15 gr.	1.6	
8 A.M.	15 gr.	1.9	
12 noon	Discontinued	2.1	Carbon dioxide combining power 67 volumes per cent
4 P.M.		2.0	
8 P.M.		0.8	
12 midnight		0.5	
4 A.M.		0.1	
8 A.M.		Trace	
12 noon		Not done	Carbon dioxide combining power 69 volumes per cent

A white male, aged 49 years, weighing 185 pounds, who for the past five years had been under treatment for pernicious anemia, was the subject in the fourth study. During the preceding eight months he had received no hydrochloric acid and only parenterally administered liver extract. Just before the experimental observations his red blood cell count was 4,010,000; Newcomer hemoglobin, 11.1 Gm.; white blood cell count, 7,300. The nonprotein nitrogen was 35 mg. per cent, and the blood sugar was 93 mg. per cent. Fifteen cubic centimeters of the solution of sulfanilamide were administered every four hours. Sulfanilamide determinations were made on the blood one-half hour before the drug was given. Immediately following the last sulfanilamide determination the stomach contents showed neither free nor combined hydrochloric acid. The results obtained are given in Table VIII.

TABLE VIII

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 75 volumes per cent
12 noon	15 gr.	0.5	
4 P.M.	15 gr.	0.9	
8 P.M.	15 gr.	1.2	
10 P.M.*	Discontinued	1.9	Carbon dioxide combining power 69 volumes per cent

*Further observations were not possible because the patient found it necessary to leave the hospital.

The subject in the fifth study was a white female, 24 years old, weighing 105 pounds. At the start of this experiment she had a red blood cell count of 4,060,000, a hemoglobin of 11.6 Gm. (Newcomer method), and a white blood cell count of 6,200; her hemogram at

this time was essentially normal. Her nonprotein nitrogen, blood sugar, and urinalysis were well within the normal limits both before and immediately following the experiment. An arbitrary dose of 60 c.c. of the solution of sulfanilamide per day was administered in divided doses, 10 c.c. every four hours. Sulfanilamide determinations were done on the blood one-half hour before the drug was administered. All determinations were done immediately at approximately 25° C.

TABLE IX

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 73 volumes per cent
12 noon	10 gr.	0.4	
4 P.M.	10 gr.	0.7	
8 P.M.	10 gr.	1.2	
12 midnight	10 gr.	1.6	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	3.1	
12 noon	Discontinued	3.2	Carbon dioxide combining power 67 volumes per cent
4 P.M.		3.4	
8 P.M.		2.7	
12 midnight		2.1	
4 A.M.†		Not done	
8 A.M.		1.1	
12 noon		0.8	Carbon dioxide combining power 69 volumes per cent
6 P.M.		0.4	

*These specimens were drawn at approximately the time indicated subject to thirty minutes' variations.

†Specimens were not drawn at these hours.

One week was allowed to elapse between the close of this series and the beginning of the next. A dose of 60 grains a day of a brand of sulfanilamide tablets (5 gr.) accepted by the Council was administered in divided doses as previously. Neither the blood chemistry nor the hematology at this time showed any significant variance from that immediately preceding the following experiment (Table X).

TABLE X

SULFANILAMIDE TABLETS

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 71 volumes per cent
12 noon	10 gr.	0.2	
4 P.M.	10 gr.	0.5	
8 P.M.	10 gr.	0.9	
12 midnight	10 gr.	1.3	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	2.2	
12 noon	Discontinued	2.7	Carbon dioxide combining power 65 volumes per cent
4 P.M.		2.9	
8 P.M.		2.4	
12 midnight		1.8	
4 A.M.†		Not done	
8 A.M.		0.8	
12 noon		0.6	Carbon dioxide combining power 67 volumes per cent
6 P.M.		0.3	

*See footnote under Table IX.

†See footnote under Table IX.

In the sixth study the subject was a white male, 41 years old, weighing 138 pounds. At the start of this experiment he had a red blood cell count of 4,320,000, a hemoglobin of 14.3 Gm. (Newcomer method), and a white blood cell count of 9,100. His hemogram was normal, his nonprotein nitrogen was 27 mg. per cent, his blood sugar was 82 mg. per cent, and his vitamin C determination was 1.13 mg. per cent; a basal metabolism run three weeks prior to this test was plus 23. A dose of 60 c.c. of the solution of sulfanilamide a day was administered in divided doses, 10 c.c. every four hours. Determinations were done as previously.

TABLE XI

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 76 volumes per cent
12 noon	10 gr.	0.3	
4 P.M.	10 gr.	0.7	
8 P.M.	10 gr.	1.4	
12 midnight	10 gr.	1.9	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	2.9	
12 noon	Discontinued	3.4	Carbon dioxide combining power 67 volumes per cent
4 P.M.		3.1	
8 P.M.		1.8	
12 midnight		1.1	
4 A.M.†		Not done	
8 A.M.		Trace	
12 noon		Not done	Carbon dioxide combining power 69 volumes per cent

*See footnote under Table IX.
†See footnote under Table IX.

TABLE XII

SULFANILAMIDE TABLETS

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 69 volumes per cent
12 noon	10 gr.	0.2	
4 P.M.	10 gr.	0.5	
8 P.M.	10 gr.	0.9	
12 midnight	10 gr.	1.3	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	2.0	
12 noon	Discontinued	2.3	Carbon dioxide combining power 58 volumes per cent
4 P.M.		1.8	
8 P.M.		1.1	
12 midnight		0.7	
4 A.M.†		Not done	
8 A.M.		0.3	
12 noon		Not done	Carbon dioxide combining power 61 volumes per cent

*See footnote under Table IX.
†See footnote under Table IX.

A period of one week was allowed to elapse between the close of this series and the beginning of the next study. A dose of 60 grains a day of a brand of sulfanilamide tablets (5 gr.) accepted by the Council was administered in divided doses as before. The blood chemistry and hematology of this subject showed no significant changes from those run before the experiment with the exception of the vitamin C determination, which was

0.72 mg. per cent. The individual in the seventh study was a 21-year-old white male weighing 149 pounds. His red blood cell count immediately preceding this experiment was 4,720,000, with a Newcomer hemoglobin of 15.8 Gm. His white blood cell count at this time was 7,600, and his hemogram was normal. His nonprotein nitrogen was 26 mg. per cent, his blood sugar was 92 mg. per cent, and his vitamin C determination was 1.26 mg. per cent. A dose of 60 c.c. of the solution of sulfanilamide a day was administered in divided doses. Sulfanilamide determinations were done on the blood one-half hour before each dose of the drug was administered. All determinations were done immediately and at approximately 25° C.

TABLE XIII

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 82 volumes per cent
12 noon	10 gr.	0.3	
4 P.M.	10 gr.	0.8	
8 P.M.	10 gr.	1.1	
12 midnight	10 gr.	1.3	
4 A.M.	10 gr.	Not done	
8 A.M.	10 gr.	2.5	
12 noon	Discontinued	2.6	Carbon dioxide combining power 78 volumes per cent
4 P.M.		2.0	
8 P.M.		1.4	
12 midnight		0.6	
4 A.M.		Not done	
8 A.M.		0.1	Heavy trace
12 noon		Not done	Carbon dioxide combining power 79 volumes per cent Vitamin C determination 0.62 milligrams per cent

A period of one week was allowed to elapse between the foregoing experiment and the following. Neither the blood chemistry nor the hematology showed any significant changes from that at the start of this experiment. A dose of 60 grains a day of a brand of sulfanilamide tablets (5 gr.) acceptable to the Council was administered in divided doses of 10 grains every four hours, as previously. Sulfanilamide determinations were done on the blood one-half hour before each dose of the drug was administered, with the exception of the 4 A.M. dose, at which time no blood was drawn for a determination.

TABLE XIV

SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 81 volumes per cent
12 noon	10 gr.	0.3	
4 P.M.	10 gr.	0.6	
8 P.M.	10 gr.	1.2	
12 midnight	10 gr.	1.4	
4 A.M.	10 gr.	Not done	
8 A.M.	10 gr.	1.9	
12 noon	Discontinued	2.2	Carbon dioxide combining power 72 volumes per cent
4 P.M.		1.7	
8 P.M.		0.9	
12 midnight		0.4	
4 A.M.		Not done	
8 A.M.		Very faint trace	
12 noon		Not done	Carbon dioxide combining power 79 volumes per cent

The blood chemistry showed no significant changes at this time over that preceding this experiment; the red blood cell count and hemoglobin were approximately the same; the white blood cell count was 4,800, with a normal hemogram.

A period of one week was allowed to elapse between this experiment and the following study. The patient's blood chemistry immediately preceding this experiment was essentially the same as previously. The white blood cell count was 9,200. Sixty cubic centimeters of sulfanilamide solution, with concomitant dosages of sodium bicarbonate, were administered as previously.

TABLE XV

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE PLUS SODIUM BICARBONATE

TIME	DOSAGE	MILLIGRAM PER CENT	URINE
8 A.M.	10 gr.	0.0	Alkaline
12 noon	10 gr.	0.2	Alkaline
4 P.M.	10 gr.	0.6	Alkaline
8 P.M.	10 gr.	1.3	Alkaline
12 midnight	10 gr.	1.5	Alkaline

Tests were discontinued because of patient's refusal to cooperate further.

The individual in the eighth study was a white female, aged 51 years, weighing 109 pounds, who for nine years had been under treatment for pernicious anemia. At this time, her red blood cell count was 3,940,000, with a Newcomer hemoglobin of 12.2 Gm. Her white blood cell count was 6,600, with a normal hemogram. The nonprotein nitrogen and blood sugar values were well within the normal limits. A basal metabolism run five weeks prior to this test was plus 9. A modified G-I series showed a rather marked increase in stomach motility, and the gastric analysis showed a total achlorhydria.

An arbitrary dose of 60 c.c. of solution of sulfanilamide a day was administered in divided doses every four hours (10 c.c.). Sulfanilamide determinations were made on the blood one-half hour before the drug was administered. At the close of this experiment, a nasal tube was passed and the gastric analysis still showed a total lack of free and combined acid. At this time this test was discontinued because the patient felt dizzy and complained of a headache; the following day, although she refused further laboratory studies, she reported that these symptoms had all disappeared.

TABLE XVI

TIME	DOSAGE	MILLIGRAM PER CENT
8 A.M.	10 gr.	0.0
12 noon	10 gr.	0.4
4 P.M.	10 gr.	0.9
8 P.M.	10 gr.	1.5
10 P.M.		2.3

DISCUSSION

The results obtained in this study are summarized graphically in Table XVII. It will be seen that the maximum concentration of sulfanilamide attained in the blood in every case was higher when the drug was given in the form of the solution. With sulfanilamide tablets, excretion time was shorter, and the per cent loss of blood carbon dioxide combining power was greater.

In the face of a known, persistent, complete achlorhydria, a rise in blood sulfanilamide, comparable to that obtained in normal subjects, occurred when the drug was administered in the form of a solution.

When the solution of sulfanilamide was administered, together with large doses of sodium bicarbonate (20 to 30 gr. every two hours), no interference with absorption of the sulfanilamide occurred.

TABLE XVII

SUBJECT			TOTAL SULFANILAMIDE ADMINISTERED	MAXIMUM CONCENTRATION REACHED (MG.%)	TIME FOR EXCRETION (HOURS)	CO_2 AT START (VOL. %)	CO_2 AT MAXIMUM CONCENTRATION (VOL. %)	CO_2 AT FINISH (VOL. %)	PERCENTAGE OF LOSS IN CO_2 FROM START TO MAXIMUM CONC.
1 Male 26 years 190 pounds	Solution of sulfanilamide		105	2.9	24	63	57	61	0.095
	Sulfanilamide tablets N.N.R.		105	2.4	24	67	54	58	0.194
	Solution of sulfanilamide and sodium bicarbonate	*	105	2.9		68	84	86	
2 Male 30 years 194 pounds	Solution of sulfanilamide		105	2.8	24	71	69	67	0.028
	Sulfanilamide tablets N.N.R.		105	2.0	20	68	61	58	0.103
3 Male 28 years 181 pounds	Solution of sulfanilamide		105	2.3	20	76	72	67	0.054
	Sulfanilamide tablets N.N.R.		105	2.1	16	73	67	69	0.082
4 Male 49 years 185 pounds	Solution of sulfanilamide	†	60	1.9		75	69		0.080
5 Female 24 years 105 pounds	Solution of sulfanilamide		70	3.4	24	73	67	69	0.082
	Sulfanilamide tablets N.N.R.		70	2.9	24	71	65	67	0.084
6 Male 41 years 138 pounds	Solution of sulfanilamide		70	3.4	20	76	67	69	0.118
	Sulfanilamide tablets N.N.R.		70	2.3	20	69	58	61	0.157
7 Male 21 years 149 pounds	Solution of sulfanilamide		70	2.6	20	82	78	79	0.048
	Sulfanilamide tablets N.N.R.		70	2.2	20	81	72	79	0.111
	Solution of sulfanilamide and sodium bicarbonate	*	50	1.5					
8 Female 51 years 109 pounds	Solution of sulfanilamide	‡	40	2.3					

*Twenty to 30 grains of sodium bicarbonate administered every two hours.

†Pernicious anemia, five years. Stomach contents showed neither free nor combined hydrochloric acid.

‡Pernicious anemia, nine years. Marked high motility. Gastric analysis showed total achlorhydria before and after this experiment.

CONCLUSIONS

In the human subject higher blood concentrations of free sulfanilamide are obtained when the drug is given as the solution than when administered in equivalent dosage in the form of tablets.

The rate of elimination of the sulfanilamide is no more rapid when given as the solution than when administered in other forms.

The loss of blood carbon dioxide combining power is less when the solution of sulfanilamide is administered than when the drug is given in tablet form.

Even in the face of a persistent, complete achlorhydria, a rise in blood concentration of sulfanilamide comparable to that seen in the normal subject is attained when the drug is administered in the form of the solution. Hence the influence of gastric hydrochloric acid is not required to permit absorption of the sulfanilamide into the blood stream. If a linkage between the glucose and sulfanilamide does exist, gastric acidity is not needed for its breakdown.

REFERENCES

1. Bratton, A. D., and Marshall, E. K.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537, 1939.
2. Siebert, W. J., and Loose, Frederick: Observations on a Sulfanilamide Solution, J. LAB. & CLIN. MED. **25**: 1062, 1940.

LABORATORY METHODS

GENERAL

SIMULTANEOUS ARTERIAL AND VENOUS BLOOD CULTURES*

MAYNARD MURRAY, M.D., AND FRANK MOOSNICK, B.S.
CINCINNATI, OHIO

IN REVIEW of the literature on blood cultures one is impressed by the fact that a comparative study of the efficiency of arterial blood cultures as compared to venous is wanting. In cases of septicemia it is not uncommon to see petechial hemorrhages follow the distribution of a small artery. Because of these facts such a comparative study was conducted on 27 patients with clinical evidence of bacteriemia.

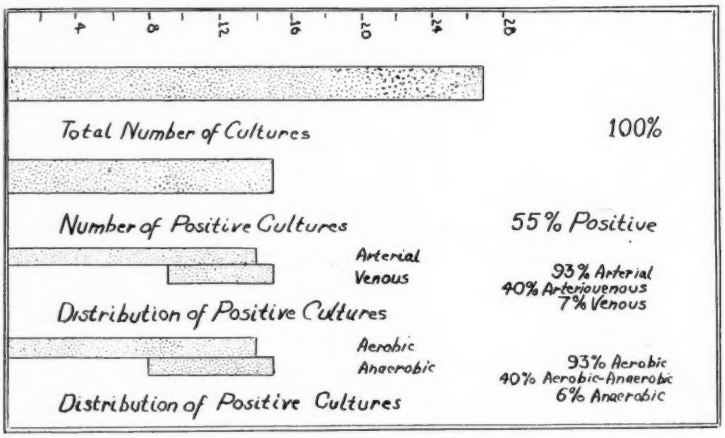


Fig. 1.

After surgical cleansing of the parts blood cultures were taken simultaneously from the cubital vein and from the femoral artery. The medium used was nutrient brain broth. The cultures were incubated at 37° C. for six days and subcultures were taken. The blood taken from each source was about 5 c.c., half of which was cultured aerobically and the other half anaerobically. As seen in Fig. 1 the results are as follows:

From 27 cultures taken from as many patients 15, or 55 per cent, were positive. In the 15 positive cases 14 were positive in the arterial culture, while only 6 were positive in the venous culture. One patient showed a positive venous culture and a negative arterial culture. As to the distribution of aerobic and anaerobic cultures, 15 aerobic and 7 anaerobic cultures were positive. One culture was positive in the anaerobic tube and negative in the aerobic tube.

From this experiment it is evident that arterial cultures are positive in a higher percentage of cases than are cultures taken from a vein.

*From the Laboratory of Physiology, University of Cincinnati and Longview State Hospital, Cincinnati.

A SIMPLIFIED APPARATUS FOR CONSTANT RATE INJECTIONS*

ALFRED E. KOEHLER, M.S., PH.D., M.D., SANTA BARBARA, CALIF.

IN STUDYING the ability of the animal body to utilize, store, or eliminate a substance injected intravenously, it is desirable to give it at a constant rate over a sufficiently long period of time, so that the organism can adapt itself and establish, if possible, an equilibrium. Similarly, in studying the effect of a substance such as a hormone upon the animal organism, the true response can usually better be evaluated if such a substance is given at a constant rate approaching physiologic or pathologic secretion.

Besides affording adequate time for diffusion and distribution equilibrium, continuous injection has the particular advantage over a single injection in that it frequently permits the observation of the change in equilibrium or response that may take place during the course of administration. Thus, if glucose is injected intravenously at a constant rate, the rapidity of disappearance of glucose from the blood stream constantly changes as the mechanism for sugar utilization becomes stimulated or, as in the case of the diabetic organism, first stimulated and then depressed. Similarly, if epinephrine is injected at a constant physiologic rate for two or four hours the blood pressure response may be quite different for the consecutive half hour intervals, in fact, may change from a pressor to a depressor effect. This difference of adaptation is often of primary importance and may be completely missed by a single injection even if prolonged to one-half hour.

APPARATUS FOR INJECTION

Several types of apparatus for constant rate injections that have been satisfactory for specific purposes have been described. One of the first motor driven pumps was described by Bock[1] in 1907, and modifications have been made by Friedman[2] (1910) and many others. Considerable improvement was, however, made in compactness, mechanical precision, and adaptability by Woodyatt[3] in an apparatus described in 1917. This machine, as originally described, was subject to current fluctuations; due to the valvular mechanism involved, it never became popular for clinical use.

The apparatus to be described, although not capable of injection over indefinite periods as is the case with the Woodyatt pump, offers a simple, accurate means of constant rate injection, particularly practical for routine clinical tolerance tests. Our experience has shown that the average subject or patient cannot lie longer than two and one-half to three hours for injection without becoming unduly restless or uncomfortable, and consequently an apparatus for longer injection is usually not needed.

*From the Sansum Clinic and Santa Barbara Cottage Hospital, Santa Barbara.
Received for publication, November 29, 1939.

DESCRIPTION OF APPARATUS

Fig. 1 shows the front and side views of the apparatus. It consists essentially of a vertically supported syringe of 200 c.c. capacity or less that is actuated by a constant speed motor. The platform is about 7½ inches square, and the two front corners support rigidly 2 steel tubes ⅝ by 18 inches, 5⅛ inches apart center to center. Sliding on these tubes are the stationary but adjustable support

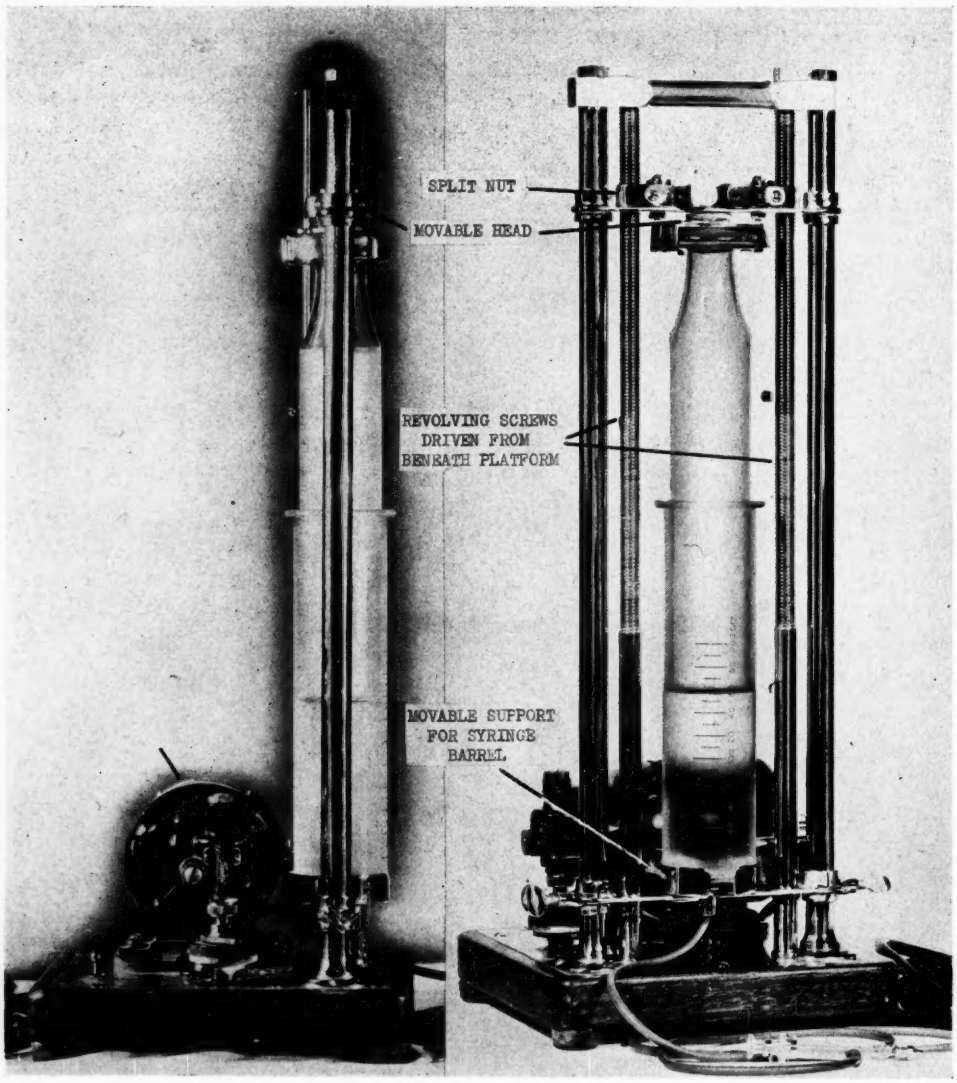


Fig. 1.

for the bottom of the barrel of the syringe and also the screw actuated head which clasps the top of the plunger. Inside of each stationary vertical support and parallel to it is a screw supported by a bearing in the top cross bar and also by a bearing in the base plate through which it extends. The screw shaft is of ½ inch diameter with 13 threads per inch. The head carrying the plunger

carries two split nuts which engage with the screws. Spring tension keeps the split nuts together to engage the thread, but pressure on the clip extensions with forefinger and thumb of each hand disengages the head so that it can be freely moved up or down on the outside supports.

The two screws are synchronously turned beneath the top plate of the base by sprockets and chain connected to the motor drive which extends through the base plate from above. The sprockets at the base of the screws are 2 inches in diameter with 20 teeth, and the sprocket on the motor drive extension shaft has 10 teeth.

A constant speed motor of 50 cycle 1,500 revolutions, $\frac{1}{90}$ horsepower, synchronous type with a 1:1,120 reduction drive extending downward is mounted on the back portion of the base.* Mounting the motor on a rubber base and inserting a rubber disk universal drive in the power extension minimizes vibration and noise.

The above type motor and reduction drive ratios result in the delivery of 100 c.c. per hour with the type of 200 c.c. syringe we have used.

An automatic shut-off switch operated when the syringe plunger is down is essential. The switch is mounted on the lower barrel support and is operated by a rod that extends up through an extension of the movable head. An adjustable collar fastened on the rod is engaged when the head has moved down sufficiently. The mounting of the switch on the lower movable support serves to maintain a constant shut-off relationship regardless of the position of the support.

Obviously, any rate of delivery can be obtained by using different drive sprocket ratios or by the introduction of various gears. Adjustable 3 or 4 speed transmissions can be built in below the base. On the other hand, we have found it desirable to make the driving mechanism as simple as possible so as to avoid mistakes.

To vary the amount of a substance to be injected it has in general proved more satisfactory to change the concentration of the solution rather than change the rate of injection of the solution. Thus glucose can be given at the rate of 10, 25, or 50 grams per hour by using a 10, 25, or 50 per cent solution injected at the rate of 100 c.c. per hour. Epinephrine or insulin can be diluted with normal saline, so that any desired amount can be given per hour.

The main advantage of this type of apparatus is its simplicity and practicability, so that it can be used for routine bedside work. The standard syringe and tubing can, of course, be easily sterilized.

REFERENCES

1. Bock, J.: Untersuchungen über die Nierenfunktion. I. Ueber die Ausscheidung der Alkalimetalle nach Injektion von Kaliumsalzen, Arch. f. exper. Path. u. Pharmakol. **57**: 183, 1907.
2. Friedman, E.: Zur Technik der Durchströmung überlebender Organe, Biochem. Ztschr. **27**: 87, 1910.
3. Woodyatt, R. T.: The Method of Timed Intravenous Injections, J. Biol. Chem. **29**: 355, 1917.

*Such a constant speed motor may be obtained from the Bodine Electric Co., Chicago, Ill.

A SIMPLE PHOTOSTATIC CAMERA

ROBB SPALDING SPRAY, PH.D., AND ALFRED REEVES STANLEY, PH.D.
MORGANTOWN, W. VA.

PHOTOSTATIC records of books, journals, or other documents are a matter of great convenience and importance, especially where reprints are no longer available, or where library loans do not allow time for abstracting or for translation of foreign texts. "Bibliofilms" now provide a fairly convenient source of such records.

To meet daily desires, we have designed and built a photostatic camera of such simplicity and general utility that it seems suitable to present its details in such form that others may reproduce it. This device prints directly upon photographic paper, 5 by 7 inches, with only a slight reduction in size of text, in sharp white type upon a solid black ground. Even the finest types are sharply and legibly reproduced. Apart from the initial cost, prints may be made in quantity at approximately 2 cents each.

The apparatus consists of a baseboard with vertical grooved standards to support a camera, with its plate holder removed, on an adjustable slide. Fitted tightly above the camera is a light-proof horizontal box carrying a mirror at 45° angle, centered on the camera lens. The mirror is necessary to re-reverse the normally reversed image. Fitted into this box is a movable frame designed to receive an Eastman printing frame, 5 by 7 inches, fitting into a recess ¾ inch deep.

This movable frame is supported on a shelf attached to the camera slide, and is arranged to slide into or out of the mirror box, allowing 1½ inches play for focusing. Guide strips and a wing-nut bolt to lock in focus are provided.

Three focusing movements are thus available: (1) the camera bellows; (2) the vertical movement of the camera slide; (3) the horizontal movement of the printing frame carrier. By proper manipulation of these movements all ordinary journal and book pages can be quickly reduced and sharply brought to focus on a 5 by 7 inch ground-glass screen.

The apparatus is easily constructed, chiefly of three-ply wood, conveniently available in large sheets. Bracing consists chiefly of 1 by 1 inch strips. Glue, wire brads, and ⅛ inch wood screws are applied as indicated. The construction details are given in the illustrations and key to details. Basic dimensions alone are given.

We are using a Folmer and Schwing camera, 4 by 5 inches, as provided by Spencer Lens Co. (1924 catalog, No. 640, page 71). This is a speed graphic bellows camera with Kodak ball-bearing shutter and a 4 by 5 R. R. lens.

For illumination of books in photographing we use two Westinghouse par-38 projection flood bulbs, 150 watt, 120 volts. These are supported in clamps on metal standards at about 18 inches from the book, directed downward at

Received for publication, December 8, 1939.

about 45° angle. With this illumination the exposure time runs around ten to twelve seconds, according to the selected printing paper.

From a variety of papers tested we have chosen Eastman Kodaline Bromide, single weight, smooth, semimatte, white paper. However, there is now available a new Kodabrom, grade A4, single-weight paper which promises even better contrast. We have not yet tried this new line, but from our experience with single-weight Kodabrom F4 paper we believe that this may be recommended.

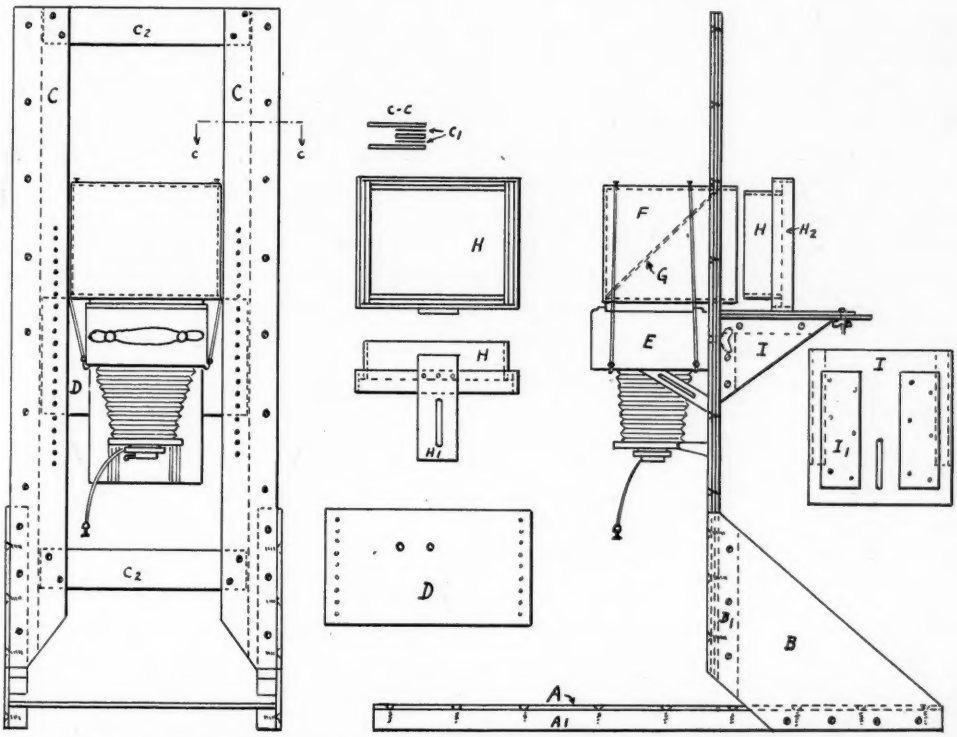


Fig. 1.—*A*, ¼ inch three-ply baseboard, 30 by 14 inches, supported on 1 by 1 by 30 inch runners (*A1*); *B*, ¼ inch three-ply gusset brace, 11½ by 12½ inches, supporting *C-C* through 1 by 1 by 9½ inch strips (*B1*); *C-C*, ¼ inch three-ply support standards, 34 inches long, constructed of two ¼ by 3 by 34 inches and one ¼ by 1½ by 34 inches, separated by two strips of cardboard (*C1*), to form groove for slide *D*; drilled with a series of ⅛ inch holes for vertical adjustment of slide D; *C2*, two ¼ by 2 by 10⅞ inch bracing strips to support *C-C*; *D*, ¼ by 6 by 10⅞ inch slide for camera support; drilled with two 5/16 inch holes to fit camera connection holes, and ends perforated by a series of ⅛ inch holes to fit similar spacing on standards *C-C*. Slide *D* held in position at desired elevation by two 6-penny casing nails. Camera attached to slide D by knurled bolts provided with camera outfit; *E*, Spencer Photo-Micrographic Camera (1924 catalog No. 640) supporting projection tunnel *F*, and clamped to it by four heavy rubber bands and wire hooks; bands looped over four brads on top of *F*; *F*, Projection tunnel, ¼ inch three-ply, 8 by 6 by 7 inches, holding *G*, silvered clear glass mirror, smooth and free of striations, 7½ by 6⅞ inches, set at exactly 45° angle, and held in place in *F* with small glued wood blocks; *H*, Focusing tunnel, fitting snugly into *F*, and movable on shelf *I*; *H2*, Collar built onto *H*, to hold standard 5 by 7 inch printing frame, allowing insertion of frame to a depth of ¾ inch. Tunnel *H* allows 1½ inch sliding insertion into *F* for focus. Tunnel *H* is guided in groove on shelf *I* by slide *H1* (¼ by 2 by 5½ inches). Slide *H1* has a 3 by ¼ inch slot to match slot on shelf *H*, fitted with 3/16 by 1 inch bolt and wing-nut to lock *H* at proper focus; *I*, Shelf, ¼ by 7½ by 8 inches, with two ¼ by 2 by 6 inch strips (*I1*) to form slide for *H1*; provided with 3 by ¼ inch slot to match *H1*.

Developing, Fixing, and Drying Details.—

1. Eastman developer, formula D-72 (about one minute).

2. Acid rinse bath (48 c.c. of 28 per cent acetic acid to 1 liter of water) (five to six seconds).

3. Eastman fixing bath, formula F1 (ten to fifteen minutes).

4. Wash at least thirty minutes in running water, or preferably until hypo test is negative (hypo test formula HT-1a). Note: Eastman chemicals are used throughout.

5. Prints are rolled and dried slowly on Ferrotype squeegee plates which are treated with "Peep-o-Day" squeegee paste (Karika Mfg. Co., Yale, Okla.), obtained through Eastman stores.

Printing suggestions.—Books or journals are laid flat on the baseboard *A*. The selected page is pressed flat under a clear sheet of window glass. The back of the book is bent out and held against a triangular rack (not illustrated) with sliding wire hooks to hold back the loose pages.

Printing frames are loaded and prints developed under the light of a Kodak safelight lamp, using a series OA Wratten safelight.

In printing a continuous series of pages we load and print six frames. With two persons working, one loads the frames, turns the pages, and manipulates the shutter; the other adjusts the focus, holds the printing frames in place, and operates the illumination switch. Working together, pages may be printed in a series at a rate of a little less than one per minute.

Complete reprint series are then conveniently bound with an Ace "pilot" stapler.

NOTE: Since the acceptance of this article for publication, we have found it more rapid to omit the use of plate holders. A glass plate is fixed in the collar *(H2)* and print paper is faced to the glass and held during printing by a felt-faced board. The printing speed is thus almost doubled.

AN OPEN RACK FOR THE SYRINGE OUTFIT WHEN IN USE

JOSEPH L. SZEKELY, M.D., FLUSHING, N. Y.

NEXT to the stethoscope, the syringe is probably the most frequently used medical instrument today. Yet the vast improvement that took place in the technical armamentarium during the last few decades has neglected to provide a commercially available, adequate solution to the simple, every-day problem of where to rest the sterile syringe outfit while in use. Before and after filling a syringe with the solution, and while it is being taken to the patient, the outfit should be placed in a secure position and the sterility of the needle and the withdrawn parts of the plunger should be safeguarded. This is somewhat difficult. The rolling tendency and the fragility of the syringe are a handicap. Various makeshift arrangements are resorted to, such as putting a piece of cotton on the tray under the tip of the syringe, placing the syringe with the needle extending over the edge of the table or tray, or even in an ash tray, etc. The disadvantages, such as the hazard of contamination, the bending of the needle point, and the possibility of breaking the syringe, are obvious and often happen.

The device described here answers this problem. It is a flat steel rack supported by two cylindrical legs with either one or three resting places for a

Received for publication, December 1, 1939.

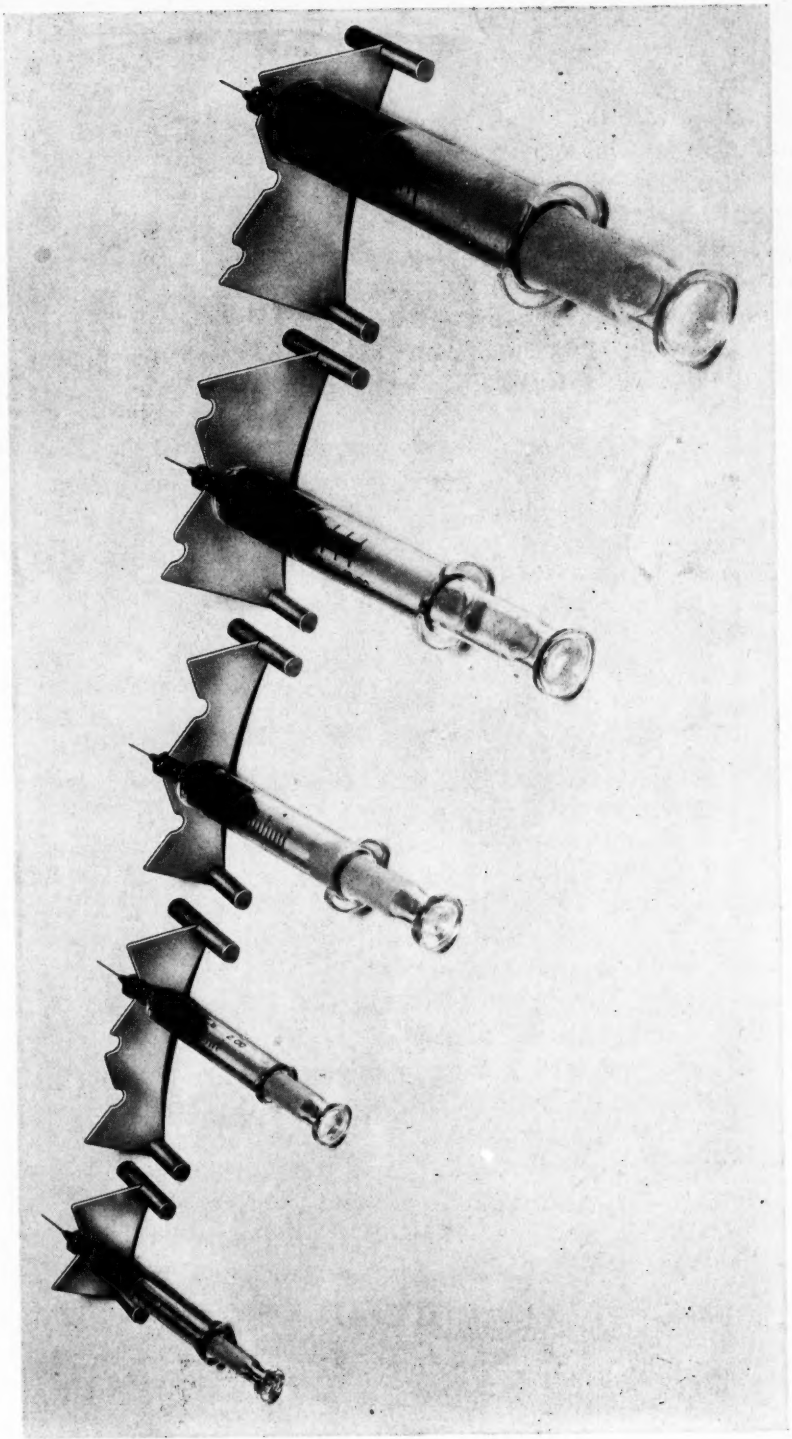


Fig. 1.

corresponding number of syringes. It is called the Steri-Rack and is made in three models. The small model will rest one syringe; the medium and the large, up to three syringes. The resting places have the shape of a semicircle that broadens out into a wide open V. The syringe outfit is usually placed in a slanting position with the hub of the needle or the tip of the syringe in the semicircle and the other end on the table or tray. The semicircle will just take the medium part of the hub of the needle. The front and rear prominences of the hub cannot pass through this opening; hence the syringe will not slip off the rack easily. If the needle happens to have a hub varying from the conventional style, the part of the syringe tip right behind the hub will rest in the semicircle with the same effect. Both glass and metal tipped syringes can be rested on the rack whether a needle is attached or not. The height of the resting levels varies to suit the different size syringes (Fig. 1).

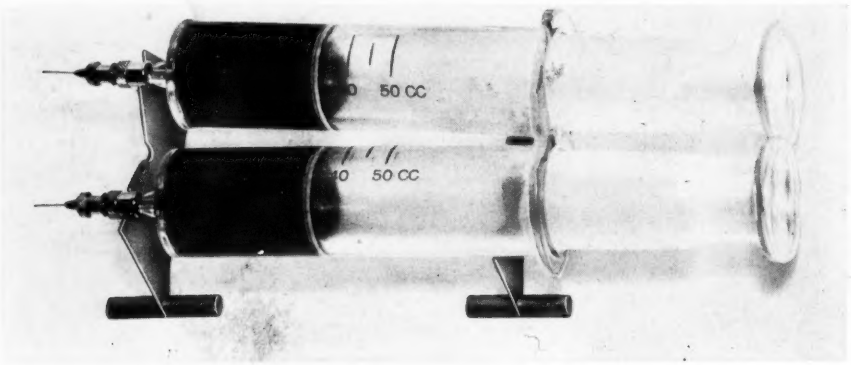


Fig. 2.

The V-shaped part of the resting place will take the barrel or the end of the plunger when a syringe is rested on two racks simultaneously (Fig. 2). The front end of the syringe outfit, either the hub or the barrel, rests on one rack, and the rear part, either the barrel or the end of the plunger, on another. Two different racks or two of the same model can be paired. The various heights of the semicircular and V-shaped resting places complement each other. This makes it possible to rest in a balanced position one or two of any size syringe, from the smallest up to a large metal ear syringe with both ends supported. Such an arrangement is advantageous, for instance, where prolonged sterility must be maintained or the barrel has a protruding side part, like that on a venoclysis syringe. The raised bottom line and the cylindrical legs, proportionate in length to the height and width of the rack, insure stability. The device is made throughout of stainless steel.

42-20 Kissena Boulevard

GONOCOCCUS COMPLEMENT FIXATION TEST

ALBERT M. DI GIOIA, A.B., SAN FRANCISCO, CALIF.

IN 1911, Schwartz and McNeal emphasized the necessity of using polyvalent antigens* in the gonococcus complement fixation tests because they believed the gonococcus belonged to a heterogeneous family. Until now, except for various research investigators, interest in the test has waned for the following reasons: first, the test would give no information in known acute or subacute cases; second, most laboratories were unable to obtain suitable antigens and positive controls; third, irregularities in the performance of the test. After a study of over 800 blood samples in this laboratory, we have attempted to eliminate some of the difficulties in the performance of the test. As a result, our experiences have been gratifying.

The following technique of the test is similar to that of Kolmer with certain modifications by this laboratory:

Solutions and Reagents.—

1. 0.85 per cent sodium chloride in distilled water.
2. 2 per cent sheep cells, defibrinated or citrated.
3. Anti-sheep hemolysin, the titer of which has been previously determined.
4. Complement—freshly pooled sera from three or more guinea pigs. Complement may prove to be one of the greatest sources of error in the test, thus making interpretations by the serologist difficult. Thomson, Park, and Hamann realized the instability of the complement and recommended the combined titration for both fixing and hemolytic powers. However, we found their procedure a somewhat laborious one. The hemolytic and fixability fractions can be assured by using the pooled sera of many guinea pigs, which when kept under optimum conditions of freezing or preservation and daily titration, will give good results.

In our laboratory pigs were bled once every week and the sera frozen in the refrigerator, 1 c.c. being placed in each of several test tubes. No complement after being thawed was returned for refreezing.

5. Patient's serum heated at 56° C. for fifteen minutes. Milky or hemolyzed sera are to be avoided.

As is recommended by Cohn, the inactivated serum is stored in the refrigerator overnight. Using freshly inactivated sera seems to give false serologic results due to increased lability of reactive substances.

Employing the use of sheep cells to remove native amboceptor is to be discouraged because of the number of anticomplementary reactions which are liable to occur. Too, large amounts of amboceptor are not easily removed by one absorption. The number of zone reactions which occur are so negligible that such technical complication is superfluous.

6. Gonococcus polyvalent antigen.

*Best polyvalent antigen available to us was donated by Parke, Davis & Co.

Received for publication, December 14, 1939.

Hemolytic titration: As bacterial antigens are rarely hemolytic, this titration may be completely omitted.

Anticomplementary titration: It is sufficient to titrate after the preparation of the antigen, and once every two months thereafter.

Antigenic titration: It is sufficient to perform this titration only once in every four to six weeks, using suitable positive control. In any case, the dose (2 units) employed in the test should be one-half to one-fourth the anticomplementary unit.

TABLE I

ANTIGEN TITRATION USING HEATED GONOCOCCUS-POSITIVE SERA

Antisera are not advisable as the antibodies vary so that the antigenic unit fluctuates according to the antiserum used. The antigenic unit is taken as the smallest amount of antigen giving complete inhibition of hemolysis. Two antigenic units are employed in the test.

ANTIGEN 0.5 C.C.	HEATED POSITIVE SERA 1:5 C.C.	COMPLEMENT 2 FULL UNITS C.C.		SENSITIZED SHEEP CELLS C.C.	
1:10	0.5	1	37° C. water	1	37° C. water Read
1:15	0.5	1	bath for 2	1	bath for one
1:20	0.5	1	hours	1	hour
1:25	0.5	1		1	
1:30	0.5	1		1	
1:35	0.5	1		1	
1:40	0.5	1		1	
1:45 and higher up to 1:500	0.5	1		1	

TABLE II

COMPLEMENT FIXATION TEST

Readings are made as positive, doubtful, or negative. Positive is denoted by the inhibition of hemolysis in one or more dilutions of serum; doubtful, by slight inhibition of hemolysis in one tube only.

TUBE	SERUM C.C.	SALINE C.C.	ANTIGEN 2 UNITS C.C.	COMPLEMENT 2 FULL UNITS C.C.		SENSITIZED SHEEP CELLS C.C.		
1	0.3	0.2	0.5	1	6° to 8° C.	1	37° C.	Read when
2	0.2	0.3	0.5	1	refrigerator	1	water	controls
3	0.1	0.4	0.5	1	for 6 to 8	1	bath	have
4	0.05	0.45	0.5	1	hours	1	for	cleared
Serum control	0.2	0.3	0.0	1		1	one hour	
Antigen control	0.0	0.5	0.5	1		1		
Positive control	0.2	0.3	0.5	1		1		
Negative control	0.2	0.3	0.5	1		1		

Complement Fixation Test.—No evaluation of the degree of positiveness was attempted, but since the gonococcus antibody is produced in most cases in small amounts, we have employed 0.3 c.c. of serum in the first tube, a larger amount than usual.

The sensitized sheep cells were prepared by mixing equal quantities of 2 per cent cells with two units of amboceptor and 1 c.c. of the mixture is added

to each tube both in the test proper and the antigenic titration. The reactions have been clearer-cut and faster.

We found that icebox incubation for six to eight hours was superior to fifteen to eighteen hours as it decreases the number of anticomplementary reactions and nonspecific positives. This may be explained by the fact that there is less destruction or absorption of complement in a shorter incubation period.

Readings of the test are made when antigen, serum, and negative controls have all cleared. This usually takes from ten minutes to twenty minutes. In this way weakly positive tests are not overlooked.

REFERENCES

Frankman, W.: Significance of Complement Fixation Test in Gonorrhea, New England J. Med. **206**: 11, 1932.

Swan, C. S.: Results and Interpretation of the Complement Fixation Test for Gonorrhea, New England J. Med. **207**: 601, 1932.

Thomson, Annis E., Hamann, Agnes C., and Park, W. H.: Irregularities in the Complement Fixation Test for Gonorrhea, J. Immunology **29**: 249, 1935.

Cohn, Alfred: The Gonococcus Complement Fixation Test, J. LAB. & CLIN. MED. 22: 627, 1937.

Kolmer, John A.: Serum Diagnosis by Complement Fixation, Philadelphia, 1928, Lea & Febiger.

Kolmer, John A.: Infection Immunity and Biologic Therapy, Philadelphia, 1925, W. B. Saunders Co.

Eagle, Harry: The Laboratory Diagnosis of Syphilis, St. Louis, 1937, The C. V. Mosby Co.

Report of the Committee for Survey of Research on the Gonococcus and Gonococcal Infection, United States Government Printing Office, 1939.

HEMATOCRIT TUBE CLEANER*

ARTHUR C. STIRLING, M.D., ROCHESTER, N. Y.

THE cleaning and drying of fine bore hematocrit tubes of the Wintrobe type have been for some years a problem to the laboratory technician, intern, or medical student. Since the use of the hematocrit tube for blood sedimentation and erythrocyte volume determinations is constantly extending, especially in the study of arthritis and in following the progress of tuberculosis and other infections, there seemed to be merit in shortening at least one step in the process. The various methods employed to date have been time-consuming, bothersome and, if the blood is caked from long sitting, sometimes inadequate. The apparatus here described shortens the cleaning process to thirty seconds and eliminates the tediousness of other methods. The cleaner is conveniently used with equipment found in practically all hospital laboratories.

The cleaner consists of a chromium plated metal tube designed as illustrated in Fig. 1. The short arm is beveled and fits into the end of a rubber tube attached to a suction system; the same apparatus employed in cleaning the ordinary hemocytometer pipette may be used. The long arm, made of smaller bore stock, is long enough to extend nearly to the bottom of a Wintrobe hematocrit tube. In position for use this cleaner arm fits loosely inside the tube.

*From the University of Rochester School of Medicine and Dentistry.
Received for publication, December 11, 1939.

Suction is applied at the beveled end which is connected to the rubber tubing. The hematocrit tube to be cleaned is slowly lowered over the long arm; the serum and packed blood cells are thus in great part removed. The cleaner with the hematocrit tube still in place is lowered into a wide-necked bottle containing water (Fig. 2). Water is sucked upward around the arm of the cleaner into the hematocrit tube and finally through the cleaner to the waste reservoir; the stream of water thus quickly removes clots of blood remaining in the tube. Agitation of the water, accomplished by repeatedly raising the tube above the surface of the water permitting bubbles of air to enter, increases the speed of the process.

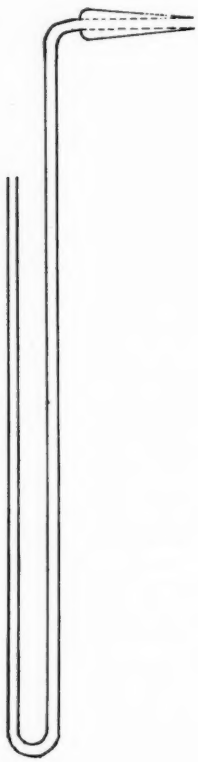


Fig. 1.

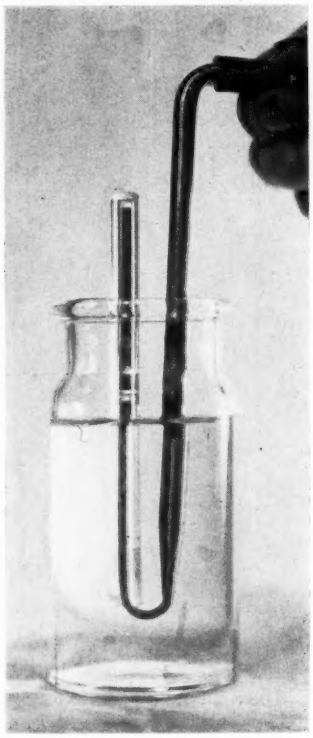


Fig. 2.

Fig. 1.—Hematocrit tube cleaner showing beveled short arm for connection with suction system.

Fig. 2.—Hematocrit tube cleaner with Wintrobe tube in place showing method of use with cleaning fluids.

Following the use of water the cleaner and tube are lowered in a similar manner, first into a bottle of alcohol which removes the excess water, then into ether which removes the alcohol. Only small amounts of alcohol and ether are needed if ample water is used. When the hematocrit tube is slowly removed from the cleaner, it is thoroughly dried upon evaporation of the ether by the air sucked into it.

Grateful acknowledgment is expressed to Mr. Raymond J. Bott of the Will Corporation, Dr. John S. Lawrence, Dr. Doran J. Stephens, and numerous others who have aided substantially in the development of this instrument.

The style of cleaner shown in Fig. 1 is available from the Will Corporation, Rochester, N. Y.

CHEMICAL

A METHOD OF COLLECTING AND TRANSPORTING SMALL BLOOD SAMPLES FOR HEMOGLOBIN DETERMINATION*

MARY E. EWING, A.B., AND N. R. BLATHERWICK, PH.D.
NEW YORK, N. Y.

THE following procedure was evolved when it became desirable to determine the hemoglobin content of the blood of certain applicants for life insurance living at a distance from the laboratory. It allows the collection and transportation of small blood samples in a suitable condition for the determination of the hemoglobin content. The method is published in the belief that others may find it useful.

Blood is collected by capillary attraction from a finger-tip puncture into a roughly calibrated glass tube from which it is transferred into an accurately graduated pipette on being received in the laboratory. The tubes are lined with a small amount of hemolytic reagent and anticoagulant which are intimately mixed with the blood just after collection by tilting the tube and allowing the blood to flow from one end to the other. The tube is then sealed at both ends with plasticine and is sent to the laboratory in a kit designed for this purpose.

The kit consists of a cardboard carton containing two cellulose acetate tubes with metallic screw caps. One tube holds a spring lancet for making the finger-tip puncture, the other carries three capillary tubes, one of which is sealed with plasticine to show how this is done, the other two are prepared for the blood collection. Only one tube is needed but an extra one is included in case of mishap to the first. The plasticine is packed into a screw cap like those used to close the cellulose acetate tubes and is inserted in a glassine envelope for packing. To seal the blood tube after completing the collection, the ends are pressed through the plasticine against the metal cap several times until about ¼ inch of the bore is compactly filled. A bundle of slides, each in a glassine envelope, is bound with a rubber band and included in the outfit if an examination of the blood film is desired. Directions for taking the blood sample are printed on a folder which is wrapped around the two container tubes when packing them in the mailing carton. The outfit is sent as first class mail and requires only 12 cents postage. Similar kits are used for blood samples taken during sugar tolerance tests and for the Kline serologic test.

The capillary collection tubes are made from the smallest bore regular glass tubing available. The tubing has an outside diameter of 3 mm. and a bore of approximately 1.4 mm. It is cut into lengths of 5¾ inches, and the ends are

*From the Biochemical Laboratory of the Metropolitan Life Insurance Co., New York.
Received for publication, March 6, 1940.

smoothed down by rubbing on emery cloth or a similar abrasive. The tubes are marked with laboratory ink 4 inches from one end, the point to which blood is collected. On receipt in the laboratory the plugged ends of the tube are filed and broken off, and the blood is transferred from the tube to a 0.1 c.c. Folin capillary pipette by placing their tips together and allowing the blood to flow into the pipette.

Potassium oxalate is used as an anticoagulant and saponin to hemolyze the blood cells. The blood must be hemolyzed when it is freshly drawn, otherwise the cells are likely to settle along the bore of the tube or at one end and cannot afterwards be as thoroughly mixed with the plasma as this method requires.

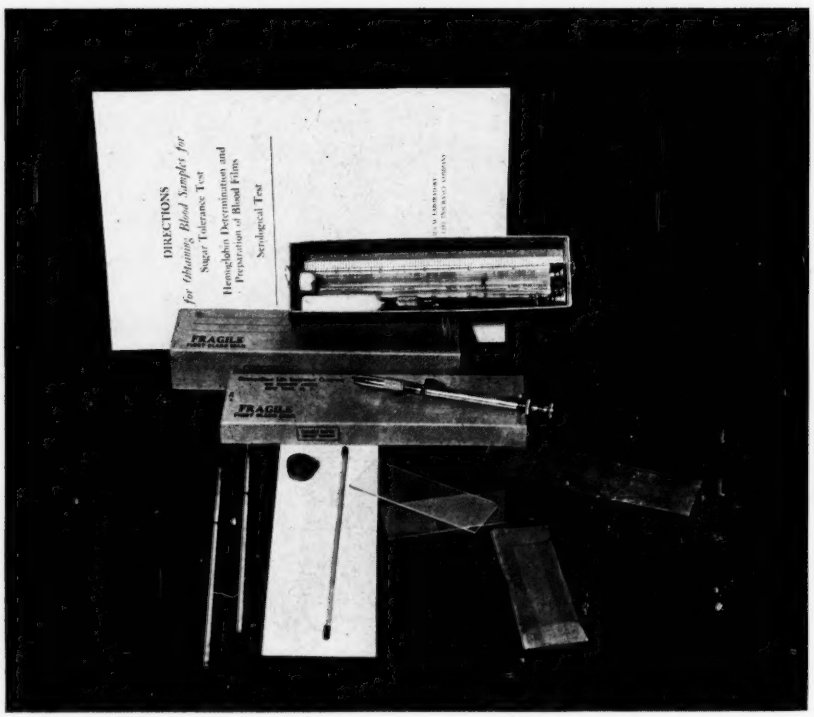


Fig. 1.—Kit for obtaining and mailing small blood samples for hemoglobin determination and examination of blood films. One cellulose acetate tube containing the blood tubes and another holding the lancet are resting in the bottom of the mailer. The inner and outer covers of the mailer are shown. The cap containing the plasticine and the specimen tube sealed with plasticine are resting on the strip of paper. The blood tubes are shown at the left of the latter. The glass slides, glassine envelopes, a lancet, and a copy of the directions are also pictured.

Between the time of collecting and analyzing the blood, a measurable amount of methemoglobin forms at the expense of the oxyhemoglobin. The analysis is, therefore, made according to Wu's cyanhemoglobin method[1] which determines both forms of hemoglobin. Oxyhemoglobin is first converted to methemoglobin by adding potassium ferricyanide, and the methemoglobin is converted to cyanhemoglobin by the addition of potassium cyanide. The same procedure is applied to a standard blood solution which has been adjusted to an oxygen capacity of 4 volumes per cent by the manometric method of Sendroy.[2] A colorimetric comparison of the blood samples with the standard gives their oxygen capacity.

PREPARATION OF THE BLOOD TUBES

One-fourth gram of potassium oxalate ($K_2C_2O_4 + H_2O$) is dissolved in 2 c.c. of water in a 50 c.c. volumetric flask and 95 per cent ethanol is added until the oxalate is precipitated.

Eight-tenths gram of saponin is mixed with a little alcohol in a small beaker until smooth and rinsed with alcohol into the 50 c.c. flask containing the oxalate. The volume is made up to the mark with alcohol.

After thorough mixing, 2 or 3 c.c. of the alcoholic suspension are poured into a small beaker. The collecting tubes are filled to the mark to which blood is to be collected, and the particles are allowed to settle along the bore. Then a fold of an absorbent towel is applied to the tip of each tube in turn and the alcohol is drawn off as nearly as possible, leaving the oxalate and saponin in the tip of the tube. A slow current of air is then blown through the tip of the tube from a rubber tube, spreading the particles in a thin film in the bore of the tube.

DETERMINATION OF HEMOGLOBIN

A quantity of blood (15 or 20 c.c.) is standardized by the oxygen capacity method and diluted with 0.4 per cent ammonia solution to an oxygen capacity of 4 volumes per cent. This solution may be kept in an amber bottle in a refrigerator for a month or two without appreciable change.

One cubic centimeter of the standard blood solution is delivered into a 20 c.c. volumetric flask and diluted with 4 c.c. of 0.4 per cent ammonia solution.

Two cubic centimeters of 0.4 per cent ammonia solution are placed in a test tube accurately graduated at 10 c.c. One-tenth cubic centimeter of blood is delivered into the tube, and the pipette is rinsed several times with the ammonia solution.

One-fourth cubic centimeter of 0.4 per cent potassium ferricyanide solution is added to the blood sample and 0.5 c.c. to the standard. After twenty minutes 0.25 c.c. of 0.1 per cent potassium cyanide solution is added to the unknown and 0.5 c.c. to the standard. At the end of five minutes both solutions are diluted to the mark with water and the unknown is read against the standard in the colorimeter:

$$\frac{20\ S}{U} = \text{Volumes per cent oxygen capacity.}$$

The cyanhemoglobin color is very stable, thus lending itself admirably to photoelectric and photometric determinations. We use the Leifo photometer made by E. Leitz, and for such readings the blood samples are diluted four times more than for colorimetric determination.

EXPERIMENTAL RESULTS

A series of 35 blood samples was kept at room temperature for periods varying from four to nine days, and the results were compared with the original values obtained on freshly drawn samples which contained neither oxalate nor saponin. The results are given in Table I. It will be seen that the figures for the preserved samples are in good agreement with those for the original samples. The values for the kept samples did not vary more than plus or minus 5 per cent

from the original values. During a period of five months we have received 108 blood samples collected by medical examiners in all parts of the country. These were entirely satisfactory for hemoglobin determination. Six samples were not collected satisfactorily. This is a good result when it is recalled that the procedure is a new one for the examiners.

TABLE I

OXYGEN CAPACITY OF FRESH AND PRESERVED BLOOD SAMPLES

SAMPLE	DAYS KEPT	OXYGEN CAPACITY IN VOLUMES PER CENT	
		FRESH SAMPLE	PRESERVED SAMPLE
1	6	20.0*	20.7*
2	6	18.8*	19.2*
3	6	18.4*	17.6*
4	6	21.3*	20.9
5	6	19.0*	18.2
6	6	21.2*	21.6*
7	6	18.4*	18.0*
8	7	19.7	20.6*
9	7	18.5	19.0*
10	7	21.3	20.9
11	7	18.5	17.9
12	7	19.4	19.0
13	7	18.0	17.9
14	7	21.7	22.2
15	7	20.0	19.5
16	7	21.4	20.8
17	7	18.7	19.1
18	7	16.8*	17.5
19	7	17.5*	17.9
20	7	17.5	17.9
21	6	17.9	17.9
22	6	18.5	18.7
23	6	20.5*	20.1
24	6	19.6*	19.7
25	6	19.4*	20.0
26	4	19.7*	20.4
27	4	19.0*	19.2
28	4	20.1	20.9
29	7	17.3	17.1
30	7	17.0	17.9
31	9	18.9	19.0
32	9	16.5	17.4
33	9	16.6	16.3
34	8	19.2	19.5
35	8	20.9	21.3

*This value is an average of duplicate determinations.

SUMMARY

A simple and inexpensive procedure is given for obtaining small blood samples which are mailed to the laboratory from all parts of the country for hemoglobin determination. Similar procedures are also used for blood samples obtained during sugar tolerance tests and for the Kline serologic test.

REFERENCES

1. Wu, H.: Studies on Hemoglobin. I. The Advantage of Alkaline Solutions for Colorimetric Methods for the Determination of Hemoglobin, J. Biochem. **2**: 173, 1922; Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Methods, Baltimore, 1932, Williams & Wilkins Co.
2. Sendroy, J., Jr.: Manometric Determination of Hemoglobin by the Oxygen Capacity Method, J. Biol. Chem. **91**: 307, 1931.

A DISTILLING APPARATUS AND A PROCEDURE FOR THE DETERMINATION OF ALCOHOL IN BLOOD AND URINE*

GEORGE W. JOHNSTON, M.S., AND R. B. GIBSON, PH.D.
IOWA CITY, IOWA

THE apparatus permits the rapid distillation of the alcohol from blood or urine and its oxidation to acetic acid with dichromate and sulfuric acid under reflux. Thus the loss of alcohol and of the intermediate oxidation product, acetaldehyde, is avoided. Ground glass connections throughout and ease in cleaning reduce the chances of contamination with organic material to a minimum. The determination is simple. The specimen, mixed in picric acid solution, is distilled (Nicloux)[1] into dichromate solution, sulfuric acid is introduced through the reflux tube for the oxidation, and the excess dichromate is titrated in the receiving flask when cool with a ferrous sulfate-methyl orange mixture (Harger).[2] Duplicate analyses check usually within 1 per cent with a maximum error of not over 2 per cent.

The apparatus, of pyrex glass, is shown in Fig. 1. Duplicate distillation flasks and three or more receiving flasks are desirable. The total cost should not exceed $50. A microburner is employed for heating. The apparatus is assembled with clamps and rings on an iron laboratory support stand. The set-up requires but little table space and may be left assembled for use.

ANALYTICAL PROCEDURE

Solutions.—1. Standard dichromate, exactly 2.1288 Gm. of potassium dichromate (analytical) are dissolved in and made up to 1,000 c.c. with distilled water.

2. A saturated solution of picric acid (recrystallized), approximately 12.5 Gm. to 1,000 c.c.

3. Titrating solution, a mixture of 60 c.c. of 50 per cent (by volume) sulfuric acid, 40 c.c. of 0.1 per cent methyl orange in N/40 sodium hydroxide, and 2 c.c. of ferrous sulfate solution (50 Gm. crystals and 30 c.c. of concentrated sulfuric made to 250 c.c.), the mixture to be prepared the day the analysis is done, and cooled to room temperature before using.

The Analysis.—The apparatus and other glassware used are to be chemically clean. Pipette exactly 10 c.c. of the dichromate solution into each of two receiving flasks and into two 250 c.c. Erlenmeyer flasks (to standardize the titration mixture). Introduce 20 c.c. of picric acid solution into each distilling flask along with a few chips of unglazed porcelain. Connect one receiving flask to the condenser. Pipette 1 c.c. of blood (after mixing the specimen) or of urine

*From the Pathological Chemistry Laboratory of the University Hospital, the State University of Iowa.
Received for publication, April 29, 1940.

into one of the distilling flasks, attach the fractionating-connecting column, mix by rotation, and connect on a support ring and gauze to the condenser. See that the ground glass joints fit properly. Distill over about 10 c.c., turn off the burner, and remove the connecting column and distilling flask. Pour slowly

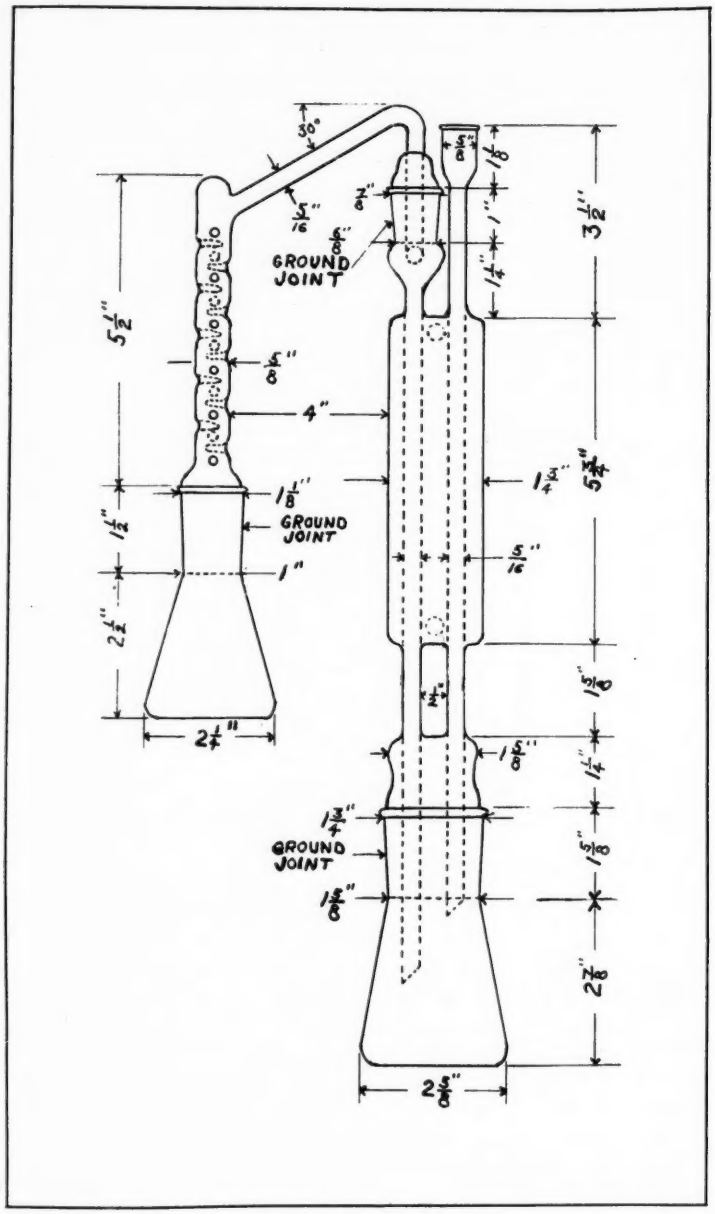


Fig. 1.

about 20 c.c. of concentrated sulfuric acid through the reflux tube of the condenser into the receiving flask and mix by lifting and rotating the set-up. The heat generated by the sulfuric acid suffices for the oxidation. After ten minutes disconnect the receiving flask and cool to room temperature. Repeat the

distillation and oxidation for a duplicate determination. Next add 10 c.c. of water to each of the blank standard dichromate flasks and 20 c.c. of sulfuric acid, and cool to room temperature. The blanks and the unknowns in the receiving flasks are titrated with the red reducing titration mixture from a burette. The color change is from an orange or yellow-green through a clear green to a solution with a slight reddish tinge. The end point is sharp and most satisfactory.

Calculation.—Ten times the distillate titration is divided by the blank titration, and the result is subtracted from 10.00. The figure thus obtained, when multiplied by 50, gives the milligrams per 100 c.c. of the sample.

The apparatus and procedure described permit certain refinements for the determination of blood and urine alcohol content which should appeal to and will be recognized by the competent analyst. Regardless of the analytical procedure employed, results which are presented as evidence in court should be checked by analysis in duplicate, a customary requirement for reliable commercial testing and for research data. The analytical method outlined above will facilitate such checking.

Acknowledgment is made to Mr. Herman Wiegand of the Department of Chemistry for suggestions as to design and for the construction of this apparatus.

REFERENCES

1. Nicloux, M., LeBreton, E., and Dontcheff, A.: Méthode de dosage de quantités d'alcool de l'ordre du dixième de milligramme, Compt. rend. Soc. de biol. **116**: 79, 1934.
2. Harger, R. N.: Simple Micromethod for Determination of Alcohol in Biologic Material, J. Lab. & Clin. Med. **20**: 746, 1935.

A RAPID SIMPLE TEST FOR SULFANILAMIDE AND ITS DERIVATIVES*

Milton M. Hartman, M.D., San Francisco, Calif.

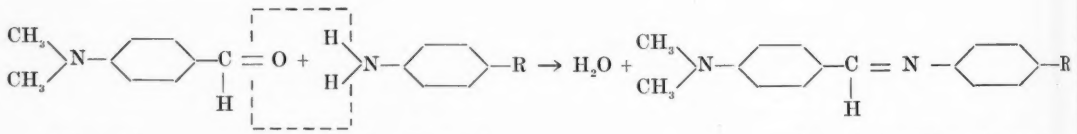
KNOWING the desirability of having an instantaneous simple test which employs only one common, cheap, and stable reagent for drugs of the sulfanilamide group, I wish to report my original application of an old and hitherto overlooked principle of organic chemistry. The reagent is one which most physicians probably already have in their offices. The test is applicable quantitatively to urine and qualitatively to other body fluids.

The tests heretofore, which are all either the original Marshall test or a modification of it, require the diazotization of the amino group on the benzene ring with nitrous acid and the coupling of the resulting compound in acid solution with dimethyl-alpha-naphthylamine (or a similar compound). An azo dye, usually red or purple, is thus formed and compared with a similarly treated standard solution in a colorimeter. This test, while very accurate and sensitive, requires four reagents, two or three intermediate filtrations, and for even a fast technician at least thirty minutes to perform all the steps and ob-

*From the Department of Medicine, Stanford University Medical School, San Francisco. Received for publication, August 2, 1940.

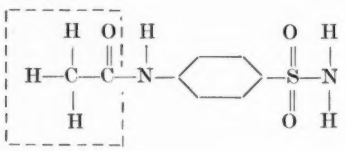
tain complete color development. All this, plus the necessity of having to make up the nitrite solution fresh each time, the expense and often nonavailability of the coupling reagent, and the inevitable high cost and delay in obtaining the desired information, result in such tests being applied much less frequently in ordinary clinical practice than they should be.

In my test the simple addition of para-dimethylaminobenzaldehyde (Ehrlich's reagent) in an acid solution to the tissue fluid produces an immediate bright yellow color. In this test the aldehyde group of the reagent reacts with the free amino group on the benzene ring of the sulfanilamide, sulfapyridine, or sulfathiazole, etc.



Reagent — Aniline Derivative — Colored Reaction Product

In the accompanying structural formula R may be any of numerous radicals in the para position. It may even be a hydrogen atom, for ordinary aniline gives a positive test. In order that the reaction may take place, it is necessary that the NH_2 (amino) group be unchanged; if even one of the H atoms is replaced by another radical, the reaction is blocked. Therefore it is a test for free (unchanged) sulfanilamide. In man these compounds are changed to a varying degree to the monoacetylated form. For example:



Para-acetylaminobenzenesulfonamide

If the total (free plus acetylated) amount of these compounds is desired, it is necessary only to heat with hydrochloric acid; in the resulting hydrolysis the acetyl group is split off and the original compound is obtained. Similarly, acetanilid ($CH_3.CO.NH.C_6H_5$) does not give the test, but when it is hydrolyzed by heat and acid with the production of aniline ($C_6H_5.NH_2$), the test is obtained.

In order to understand the application of the test and its limitations, a brief study must be devoted to possible interfering substances and their elimination. (Most of these interfere with the Marshall test also.)

1. The reagent itself is yellow, and correction must, therefore, be made for it in a quantitative test.

2. Bilirubin (bile pigment) in serum or urine imparts a yellow color to it. Dilution of the urine to 1:200 usually eliminates this interference. If the amount of bilirubin is excessive, it may be precipitated out as insoluble calcium bilirubinate by the addition of calcium chloride solution.

3. Urobilinogen in urine yields a pink color upon addition of the reagent. Dilution of the urine to 1:200 does away with this interference in practically all cases. Shaking the urine with a few drops of hydrogen peroxide solution

to oxidize the urobilinogen to urobilin (which is nonreactive) is an additional precaution; this prevents the pink color formation even in concentrated urine.

4. Other urine pigments can be disposed of by diluting the urine so that there is no perceptible yellowness and then applying the test. The urine may be diluted freely, for the compounds are detectable in a dilution of 1:5,000,000.

5. Some drugs have a free amino group attached to a benzene ring. These would rarely be present without the knowledge of the physician, the drugs in question being procaine and the antisyphilitic arsenicals, arsphenamine, neoarsphenamine, mapharsen, etc.

6. Urea yields a faint yellow color when tested with the reagent, but since the test is about 500 times more sensitive to sulfanilamide than to urea, dilution of the urine or blood filtrate disposes of this interference.

7. Drugs with an acetyl-amino group attached to a benzene ring which yield a free amino-benzene group on acid hydrolysis would interfere in the determination of total sulfanilamide. The only two compounds in this class are acetanilid and acetphenetidin ($CH_3.CO.NH.C_6H_4.O.C_2H_5$). Neither of these compounds is ordinarily administered to a patient being treated with sulfanilamide or its derivatives, the bone marrow already being subjected to sufficient hazard.

8. Sodium nitrite yields a yellow color also, but the amounts administered would rarely if ever be quantitatively significant, and the color reaction is four times more sensitive with sulfanilamide and its related compounds.

9. Alkalinity causes interference because the reaction is obtained only in acid solution. The reagent and sulfanilamide yield no color in an alkaline solution, but the yellow color appears immediately upon acidification. The reagent is, therefore, prepared in concentrated hydrochloric acid as follows:

Para-dimethylaminobenzaldehyde	1.57 Gm.
Concentrated hydrochloric acid, C. P.	20.00 c.c.
Distilled water to make	100.00 c.c.

This formula is very similar to Ehrlich's reagent, which is already universally used as a test for urobilinogen in urine. The aldehyde is much more soluble in acid solution than in neutral solution. The exact proportions of reagent and acid were arrived at by experimentation to obtain the greatest color change in proportion to the color of the reagent itself. After the addition of the reagent the liquid to be tested should be definitely acid to litmus paper. If it is not, hydrochloric acid should be added until it is. This is particularly important with blood plasma and serum which are highly buffered at a pH of about 7.4. Protein precipitation may cause some interference and necessitate filtration. Excess acidification of any fluid is to be avoided because it reduces the color response after a certain point.

10. All other usual drugs, alkaloids, inorganic salts, glucosides, etc., do not interfere. The test was applied to all the remedies mentioned in *Useful Drugs.*

QUANTITATIVE DETERMINATION OF FREE SULFANILAMIDE, SULFAPYRIDINE, AND SULFATHIAZOLE IN URINE

This method employs the Klett-Summerson Photoelectric Colorimeter with its No. 42 (blue) color filter. The results obtained agree exactly with those

obtained by the diazotization methods (after the corrections which the latter require). All figures refer to readings on this instrument with distilled water at zero, and for those who do not possess such a colorimeter the figures obtained can be used as a basis to establish a set of color standards or as a basis upon which the usual colorimeter can be used. Directions are as follows:

1. Fill a Klett tube with distilled water and adjust colorimeter to give zero reading. Discard water.

2. Prepare a 1:200 dilution of the urine. To 10 c.c. of this solution in the Klett tube add one drop of hydrogen peroxide solution and invert several times.

3. Note reading R_u. This usually runs from one to five but may go up to ten.

4. Add exactly 0.2 c.c. of reagent and invert several times.

5. Note reading R_a.

6. Calculate by use of formulas to find concentration in the diluted urine. Multiply by 200 to get concentration in original urine (undiluted).

$$\frac{R_a - R_u - 98}{7.5} = \text{Parts per million of sulfanilamide}$$

$$\frac{R_a - R_u - 98}{5.18} = \text{Parts per million of sulfapyridine}$$

$$\frac{R_a - R_u - 98}{5.06} = \text{Parts per million of sulfathiazole}$$

The figure 98 is obtained as follows: 0.2 c.c. of reagent in 10 c.c. of distilled water gives a reading of 96 with several brands of pure para-dimethylaminobenzaldehyde that the author has been able to obtain. (It might be well to check your own supply of this compound and adjust the concentration so that this figure is obtained, or to note the value that you obtain yourself.) In a 1:200 dilution of urine the urea present adds 2 to this figure, giving 98. By way of orientation it may be noted that 1:10,000 potassium dichromate (icterus index 1.0) gives a reading of 106.0. The test is most accurate with values in the diluted urine of 2 to 30 parts per million. If more is obtained, the use of a 1:400 dilution is advisable.

If a Klett colorimeter is not available, there are two other alternatives which give satisfactory results. The first is to match the color obtained with a set of permanent (stoppered) standards prepared from potassium dichromate. For example:

0.0094%	$K_2Cr_2O_7$ =	100.0 Klett =	Approx. zero p.p.m.
0.015%	$K_2Cr_2O_7$ =	159.0 Klett =	7.9 p.p.m. Sulfanilamide
0.02%	$K_2Cr_2O_7$ =	212.0 Klett =	15.0 p.p.m. Sulfanilamide
0.025%	$K_2Cr_2O_7$ =	265.0 Klett =	22.0 p.p.m. Sulfanilamide
0.03%	$K_2Cr_2O_7$ =	318.0 Klett =	29.0 p.p.m. Sulfanilamide

That is, R_a is obtained by comparison, and R_u is arbitrarily set at 2 in using the calculation formulas for the appropriate compound. The other alternative is to compare the color obtained in an ordinary colorimeter with 1:10,000 potassium dichromate, assigning to the latter the value of 106 and obtaining a value for R_a. A value of 2 can be assigned to R_u and the formulas applied.

QUANTITATIVE DETERMINATION OF TOTAL (FREE AND PARA-ACETYLATED) SULFANILAMIDE, SULFAPYRIDINE, AND SULFATHIAZOLE IN URINE

Heat 1 c.c. of urine and 1 c.c. of 2 normal hydrochloric acid in a covered beaker in a water bath (boiling) for thirty to sixty minutes. Let cool. Neutralize to litmus with 2 normal sodium hydroxide. Add water to make up to 200 c.c. Run determination as for free compound omitting the hydrogen peroxide, which is no longer necessary.

APPLICATION OF TEST TO OTHER BIOLOGIC FLUIDS (BLOOD, CEREBROSPINAL FLUID, GASTRIC CONTENTS, EXUDATES, ETC.)

The test is applicable qualitatively, but there are so many uncontrollable factors affecting the final yellowness that quantitative estimations are very unreliable. The more accurate diazotization method is to be preferred for quantitative work on such fluids. In using trichloracetic acid filtrates of blood it is found that the color response is cut down below a usable level. In trying to use ethereal and alcoholic extracts of blood, we find that the varying color and turbidity plus the yellowness of the reagent cause insuperable difficulties.

NOTE: The solution of the reagent in acid decomposes only very slowly under ordinary conditions. This change can be further minimized by storing the solution in a cold, dark place when it is not in use.

A SIMPLIFIED UREA DETERMINATION FOR ROUTINE BLOOD CHEMISTRY*

HANS NORBERT NAUMANN, M.D., LOUISVILLE, KY.

THE determination of urea in blood can be simplified by placing urease, together with oxalate, in specimen bottles, so that the blood, still warm after withdrawal, is in contact immediately with urease. The urea, converted almost instantaneously, can then be determined by direct nesslerization in Folin-Wu filtrates which at the same time serve for the common routine determinations.

TECHNIQUE

One volume of sodium oxalate and two volumes of jack bean powder are mixed by shaking in a large brown bottle which serves as stock container.† For use, the powder mixture is filled into a salt cellar with a 5 mm. opening in the cap‡ and the desired powder quantity obtained by tapping the cap of the salt cellar against the neck of the specimen bottle. (Accurate measuring is not necessary as the blood to be placed into the bottle is, as a rule, not measured either. One milligram of oxalate per 1 c.c. of blood is sufficient; 20 mg. per 10 c.c. of

*From the Long Island College of Medicine Division, Kings County Hospital, Brooklyn. The present work was continued at the Jewish Hospital, Louisville.

Received for publication, August 31, 1939.

†This powder mixture, using jack bean meal of the Arlington Chemical Co., Yonkers, N. Y., has been kept at room temperature for six months at a time.

‡Suitable are glass salt cellars with screw caps of translucent plastic in which a hole can easily be bored.

blood offers a suitable excess. With a little experience, the volume of mixed powder to be taken is easily recognized.) Immediately after placing blood into the bottle, the latter must be rotated for thirty seconds to insure solution of the oxalate and even distribution of the urease. After five minutes at room temperature the specimen is ready for precipitation: Take 3 c.c. of blood and 24 c.c. of N/12 sulfuric acid, rotate, and set aside for a few minutes. Then add 3 c.c. of 10 per cent sodium tungstate, mix thoroughly, and filter through a 12.5 Whatman No. 1 (yield: about 20 c.c.). If the precipitation is red brown instead of black brown, usually caused by too much oxalate, add a drop of concentrated sulfuric acid. The reagents are best kept in 50 c.c. burettes connected with storage bottles.

For direct nesslerization 3 c.c. of Nessler's reagent and 2 drops of 10 per cent gum acacia are placed in a test tube. Five cubic centimeters of filtrate are blown into the reagent and compared with 5 c.c. of a standard containing 0.00707 per cent of ammonium sulfate (equal to 1.5 mg. per cent N and 0.5 per cent Na_2SO_4). Holding the tip of the pipette at the edge of the test tube, blowing as forcefully as possible, and directing the jet of filtrate directly into the Nessler reagent (prepared according to Bock-Benedict) gives the clearest solutions. Gum acacia (10 per cent in 0.25 per cent benzoic acid) is added to keep the solution stable for at least thirty minutes. The addition of sodium sulfate to the standard gives the same electrolyte concentration as in the filtrate, with an identical color tinge and an identical faint haziness after standing. If necessary the 8 c.c. of unknown solution are diluted with distilled water.

TABLE I

COMPARATIVE ANALYSIS OF BLOOD SAMPLES WITH AND WITHOUT ADDITION OF JACK BEAN POWDER AFTER STANDING 3 TO 5 HOURS AT ROOM TEMPERATURE

ANALYZED FOR	METHOD	NO. OF SAMPLES ANALYZED	MEAN OF RESULTS IN MG.% JACK BEAN POWDER		MEAN OF DIFFERENCES %
			NOT ADDED	ADDED	
Glucose	Folin-Wu	125	118	125	7.1
Creatinine	Folin	52	1.91	1.90	5.5
Uric acid	Benedict	26	3.33	3.11	7.7
Chlorides	Whitehorn	21	469	469	3.2
Nonprotein nitrogen	Folin-Myers	20	36.9	41.7	30.0

Interference.—Before using the described technique generally for routine chemistry, it was necessary to investigate whether or not the addition of jack bean powder to oxalated blood interferes with the common routine tests. It will be seen from Table I that with the exception of nonprotein nitrogen determinations, the mean results obtained in samples without addition of jack bean agree within the limit of methodical error with those obtained in samples with addition of jack bean. Nonprotein nitrogen values in samples containing jack bean powder are too high and cannot be used. However, the addition of jack bean powder to oxalated blood does not interfere with the determination of glucose, creatinine, uric acid, and chlorides in the Folin-Wu filtrate.

Glycolysis.—To prevent glycolysis it is possible to use, instead of oxalate, sodium fluoride, which, on the other hand, retards the urease action. In the presence of sodium fluoride and high urea concentrations the enzymatic reaction

takes about one hour at room temperature and about half an hour in the incubator (see Table II), while in normal blood the reaction is completed in about thirty and fifteen minutes, respectively. High values obtained after twenty-four hours are due to the fact that ammonia is split off from protein as observed also by Sander.

For longer preservation of blood samples, for sugar tolerance tests, in cases of diabetes, etc., the use of sodium fluoride is necessary. If urea is to be determined in specimens containing sodium fluoride, completion of the hydrolysis of urea must be insured by allowing sufficient time for the reaction. For general routine work, however, oxalate is preferable, provided that the error due to glycolysis is minimized by precipitating the blood either after five minutes or after a certain constant time, and allowing for the decrease of glucose concentration.

TABLE II

GLYCOLYSIS AND UREASE ACTION IN OXALATED AND SODIUM FLUORIDE SAMPLES OF THE SAME BLOOD

TIME	OXALATED BLOOD AND JACK BEAN AT 20°		NaF BLOOD AND JACK BEAN AT 20°		AT 38°	
	GLUCOSE	UREA	GLUCOSE	UREA	GLUCOSE	UREA
5 min.	81	205	79	26	–	–
15 min.	76	215	–	84	81	86
30 min.	74	198	77	117	80	196
60 min.	74	195	83	197	77	215
120 min.	67	205	78	195	74	205
180 min.	68	215	77	207	79	199
24 hours	50	221	86	212	91	222
48 hours	15	228	90	235	–	–

COMMENT

The method of adding urease to oxalated blood, incubation, and subsequent direct nesslerization of the Folin-Wu filtrate was devised by Myers in 1921. This principle was adopted also by Van Slyke and Plazin, but without incubation, and substituting Somogyi's zinc* for Folin-Wu's tungstic filtration.

The technique of placing oxalate-urease powder directly in specimen bottles, as described, was used in our department during the past year and proved advantageous for the following reasons: (1) Time and labor saving. (2) Eliminates incubation, urease extracts, and buffer. (3) Rapid action of urease. (4) Clear solutions. (5) No interference with routine determinations of glucose, creatinine, uric acid, and chloride.

SUMMARY

A routine urea determination in blood has been described with the technical feature of placing an oxalate-jack bean powder mixture in specimen bottles effecting rapid hydrolysis of urea in the still body-warm blood. The urea is determined by direct nesslerization in the Folin-Wu filtrate which can be used also for other routine tests.

I am indebted to Dr. Hamilton J. Crawford, Director of the Department of Medicine at the Long Island College Division, Kings County Hospital, Brooklyn, and Dr. Carl H. Greene, Clinical Professor of Medicine, for their help and interest in the present work. I wish to thank also Miss Sylvia Held, M.A., for doing part of the analyses.

*Somogyi's copper filtrate likewise gives true sugar values and contains less substances interfering with Nessler's reaction. It is free of uric acid.

REFERENCES

1. De, P., and Bhattacharyya, S.: Glycolysis in Drawn Blood in 24 Hours, Indian J. M. Research **25**: 933, 1938.
2. Gruskin, B.: A New and Shorter Method for the Determination of Urea and Sugar in Blood, J. LAB. & CLIN. MED. **10**: 233, 1924.
3. Hawk, F. L., and Andes, J. E.: Simplified Method for Determination of Urea, Am. J. Clin. Path. Tech. Supp. **2**: 153, 1938.
4. Karr, W. G.: A Method for the Determination of Urea Nitrogen, J. LAB. & CLIN. MED. **9**: 329, 1924.
5. Looney, J. M.: The Determination of Blood Urea Nitrogen by Direct Nesslerization, J. Biol. Chem. **88**: 184, 1932.
6. Myers, V. C.: Practical Chemical Analysis of Blood, St. Louis, 1921, The C. V. Mosby Co., p. 35.
7. Sander, F. V.: The Preservation of Blood for Chemical Analysis, J. Biol. Chem. **58**: 1, 1923.
8. Seljeskog, S. R., and Cavett, J. W.: Blood Urea, Comparison of Methods, J. LAB. & CLIN. Med. **23**: 194, 1937.
9. Shapiro, C. S.: A Direct Nesslerization Method for Urea Nitrogen in Blood Where Commercial Urease Is Employed, J. LAB. & CLIN. MED. **19**: 649, 1934.
10. Van Slyke, D. D., and Plazin, J.: Peters and Van Slyke, Quantitative Clinical Chemistry **2**: 558, 1932.
11. Somogyi, M.: The Use of Copper and Iron Salts for the Deproteinization of Blood, J. Biol. Chem. **90**: 725, 1931.
12. Taylor, W. F., and Blair, W. M.: An Improved Method for the Determination of Blood Urea Nitrogen by Direct Nesslerization, J. LAB. & CLIN. MED. **17**: 1256, 1932.
13. Wrenn, H. T.: Rapid and Accurate Methods for the Determination of Urea Nitrogen in Blood and Urine by Direct Nesslerization, J. LAB. & CLIN. MED. **22**: 1040, 1936.

MODIFIED TECHNIQUE FOR PROTHROMBIN DETERMINATION IN BLOOD*

H. S. HOLMBOE, M.D., AND R. W. HOLMBOE, B.A., SANTA BARBARA, CALIF.

THE tests for prothrombin content of blood in general use are those of Quick, Brinkhous, and Smith. The determination of prothrombin by the technique of Brinkhous and Smith involves many reagents and is difficult to perform. Quick, however, has evolved a method which is simple, accurate, and inexpensive, but there are several objections to his technique; namely, the reagents must be made up fresh for each determination, the concentration of thromboplastin is variable, and the normal time value for the method is too short. This favors error in reading and does not permit the measurement of prothrombin levels above normal.

It was felt that these objections to Quick's method were valid and that a modified test might be evolved which would overcome them. Early in our experimental work we investigated several sources of thromboplastin, namely, dried brain tissue from the rat, guinea pig, rabbit, sheep, and man. The variation in results obtained with these sources of thromboplastin is shown in Table I. All tests were performed according to the technique of Quick.[12]

Subsequently the effect of varying the concentration of thromboplastin, plasma, and calcium chloride in different combinations was determined. These findings, as shown in Table II, may be summarized as follows: (1)

*From the Department of Pathology, Santa Barbara Cottage Hospital.
Received for publication, November 27, 1939.

Increasing the proportion of plasma decreases the prothrombin time. (2) Increasing the proportion of thromboplastin decreases the prothrombin time except in the case of thromboplastin from the rat. (3) There is an optimum concentration of calcium chloride for each type of thromboplastin. Increasing or decreasing this concentration prolongs the prothrombin time. (4) There is an optimal concentration of all reagents for each type of thromboplastin.

TABLE I

AVERAGE VALUES ARE GIVEN WITH STANDARD DEVIATIONS FOR EIGHT BLOOD SAMPLES

THROMBOPLASTIN FROM	PROTHROMBIN TIME (SECONDS)
Rat brain	87 ± 2.8
Guinea pig brain	201 ± 10.3
Rabbit brain	32 ± 0.9
Sheep brain	118 ± 6.2
Human brain	72 ± 1.8

The tests were made with optimum concentration of reagents (see Table II).

After a series of preliminary experiments and primarily because of its stability, an ether-soluble, acetone-insoluble extract of sheep brain was chosen as a thromboplastic agent for use in a modified technique. Its preparation is described subsequently.

MODIFIED TECHNIQUE

1. Plasma from oxalated blood is used. A mixture of 4.5 c.c. of blood and 0.5 c.c. of sodium oxalate gives sufficient plasma for several determinations. To separate the plasma from the cells, the blood is centrifuged for fifteen to twenty minutes at high speed to throw down as much of the tissue debris and as many cellular elements as possible.

2. Into a small glass tube (serology tubes 3 by $\frac{9}{16}$ inches and 4 by $\frac{1}{2}$ inches are adequate) 0.2 c.c. of plasma is accurately pipetted with a Kahn pipette. To this is added 0.2 c.c. of the thromboplastin solution. (Stock solution is diluted with normal saline 1:5 on day of test.)

3. The mixture is incubated at 37° C. in the water bath for seven minutes.

4. The tube is then removed from the water bath, the outside of the tube is dried, and exactly 0.2 c.c. of calcium chloride is *immediately* added.

5. The tube is then shaken to insure adequate mixing. It is slanted and rotated at fifteen-second intervals. The interval between the addition of the calcium chloride and clot formation is measured with a stop watch. This constitutes the prothrombin time.

REAGENTS

1. *Sodium Oxalate:* 1.34 Gm. of C. P. anhydrous sodium oxalate are dissolved in 100 c.c. of distilled water. This solution is tenth-molar and is nearly isotonic.

2. *Thromboplastin Stock Solution* (ether-soluble, acetone-insoluble brain extract): A fresh sheep brain is washed in water. The meninges and blood vessels are carefully removed. The brain tissue is then cut into pieces as small as possible and is dried with acetone. Repeated additions of acetone and grinding in a mortar facilitate this process. The final product is freed of acetone by centrifuging, carefully decanting the acetone, and drying the powder in the

TABLE II

TESTS WERE RUN BY QUICK'S METHOD. ALL RESULTS INDICATE AVERAGES AND STANDARD DEVIATIONS OF AT LEAST EIGHT DETERMINATIONS

RATIO					PROTHROMBIN TIME (SECONDS) AND SOURCE OF THROMBOPLASTIN				
NO.	PLASMA	THROMBO-PLASTIN	CALCIUM CHLORIDE		RAT	GUINEA PIG	RABBIT	SHEEP	MAN
1	1	1	1	M/10	242 ± 5.4	525 ± 9	65 ± 2.5	576 ± 19.1	153 ± 7.4
2	2	1	1	M/10	137 ± 3.4*	296 ± 27	40 ± 1.4	259 ± 12.7	109 ± 4.8
3	0.5	1	1	M/10	593 ± 17	1094 ± 16.3	109 ± 7.7	1110 ± 140	309 ± 8.5
4	1	2	1	M/10	349 ± 17†	398 ± 2.3	46 ± 1.2	436 ± 16	158 ± 8.8
5	1	0.5	1	M/10	251 ± 6.4	1124 ± 144	71 ± 1.8	740 ± 56	168 ± 6.4
6	1	1	2	M/10	560 ± 24.6‡	2080 ± 149	161 ± 3.8	1380 ± 97	432 ± 11.7
7	1	1	1	M/20	157 ± 1.9	291 ± 19	32 ± 0.9	248 ± 11.7	75 ± 3.66
8	1	1	1	M/40	109 ± 1.6	201 ± 10.3§	41 ± 1.8	138 ± 3.8	72 ± 1.8
9	1	1	1	M/80	94 ± 2.6	243 ± 18.7	34 ± 0.2	143 ± 3.8	73 ± 3.4
10	1	1	1	M/160	88 ± 3.3	290 ± 7.8	32 ± 0.9	118 ± 6.2§	120 ± 15.3
11	1	1	1	M/320	87 ± 2.8§	320 ± 11.8	35 ± 1.1	185 ± 27.2	158 ± 19.2
12	1	2	1	M/20	224 ± 7.2	297 ± 15	48 ± 2.1	154 ± 7.2	94 ± 8.1
13	2	1	1	M/80	90 ± 3.2	220 ± 10.6	42 ± 3.2	233 ± 28.4	94 ± 4.4

*Note effect of doubling quantity of plasma. (Compare lines 1 and 2.)
†Note effect of doubling thromboplastin. (Compare lines 1 and 4.)
‡Note effect of doubling calcium chloride. (Compare lines 1 and 6.)
§Note optimum concentration of calcium chloride. This optimum is only apparent with rat, guinea pig, and sheep thromboplastin. With rat, rabbit, and human thromboplastin note the range in which the concentration of calcium chloride has but slight effect (indicated by brackets).

open air. The dry powder is then completely extracted with repeated small portions of ether until no precipitate is obtained by addition of acetone. To the combined portions of the ethereal extract, acetone is added until no more of the extracted material can be precipitated from the solution. The precipitated substance is separated from the ether-acetone mixture by centrifugation. It is then air-dried and kept in a stoppered vial in the icebox. The finished product is a light brown, waxy substance which darkens on standing. A normal yield from an average sheep brain is about 5 to 10 Gm. In the preparation of the stock solution 0.25 Gm. of the substance is emulsified in 50 c.c. of normal saline, and aqueous merthiolate is added as a preservative to a concentration of 1:100,000. The saline suspension is warmed to 56° C. for ten minutes to facilitate mixing, and during this time it is thoroughly shaken. The finished product is a homogeneous opalescent mixture which for test purposes is diluted 1:5 with normal saline. We have kept the stock preparation in the refrigerator for as long as three to five months without the slightest evidence of deterioration. Brief experiments with a similar extract from rabbit brain revealed that it gave practically the same values for prothrombin time as did thromboplastin from the sheep brain.

3. *Calcium Chloride:* 1.11 Gm. of C. P. anhydrous calcium chloride is dissolved in 800 c.c. of distilled water, giving a final concentration of eightieth-molar.

All reagents except the thromboplastin are stored at room temperature.

DISCUSSION

The essential factors involved in such a test are multiple, each in turn having its separate influence upon the clotting phenomenon. Any test concerning the clotting mechanism must attempt to keep the variability of these factors at a minimum. The following techniques have been found most important and difficult to standardize:

The proportion of oxalate to whole blood is very important, and the ratio of 1:10 should be strictly adhered to. Experimentally, doubling the quantity of oxalate will increase the prothrombin time 200 per cent. Varying differences in prothrombin times may be obtained by concentrations between these extremes. Slight hemolysis in the sample of blood does not alter the prothrombin time appreciably, but gross hemolysis does shorten it sufficiently to invalidate the determination.

Although it is desirable to determine the prothrombin time of a sample as soon as possible, oxalated whole blood will usually keep satisfactorily in the icebox for twenty-four hours without material alteration in the level of prothrombin if the plasma is not separated from the cells. Individual samples of blood have been kept as long as seventy-two hours, with an alteration in prothrombin content of only about 15 per cent. Recent work in connection with blood stored in "blood banks" indicates that most blood shows a marked deterioration of prothrombin within twenty-four hours and that it is almost depleted in forty-eight to seventy-two hours. The rate of destruction or fixation is probably an individual characteristic and cannot be predetermined.

It is important that the concentration of all reagents, and particularly neutral salts, be maintained at a uniform level. Normal saline must be used

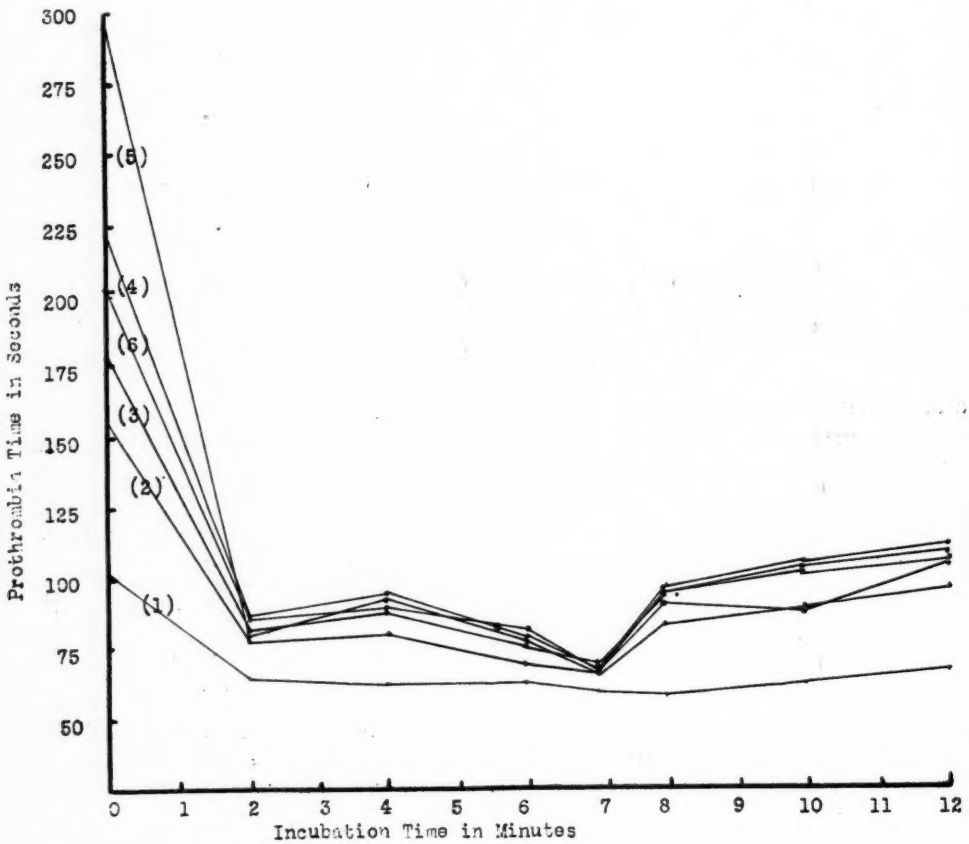
in the thromboplastic solution because an excess of sodium chloride will materially delay the clot formation. This phenomenon is not universal, and individual samples of blood vary in their susceptibility to this change.

TABLE III

TEST AS OUTLINED, USED IN ALL CASES

INCUBATION PERIOD (MINUTES)	PROTHROMBIN TIME (SECONDS) CASE						SPREAD	AVERAGE
	1	2	3	4	5	6		
0	101	153	176	222	290	202	121	175.6
2	66	78	82	86	80	85	20	79.5
4	65	82	88	95	93	89	30	85.3
6	65	71	77	80	79	83	18	74.3
7*	61	67	74	72	69	70	13*	68.8*
8	58	79	87	92	84	86	34	82.6
10	65	88	99	102	87	96	37	89.5
12	68	93	104	108	94	98	40	94.2

*Note minimum spread and shortest prothrombin time.

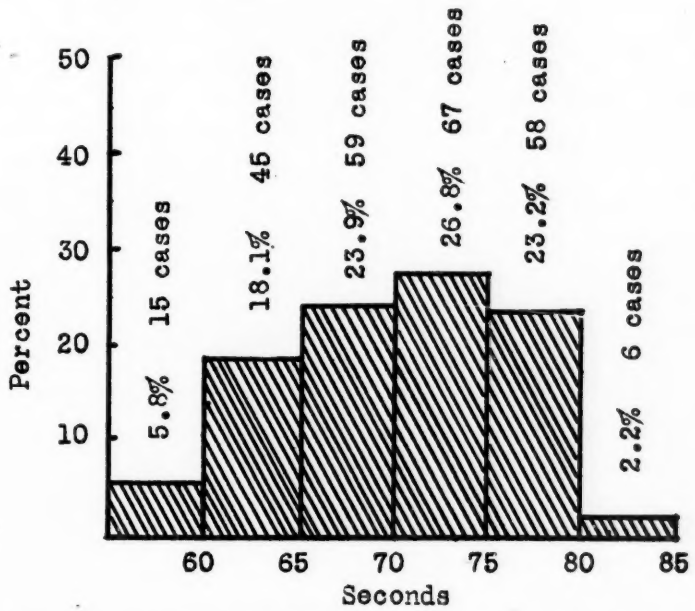


In the reading of the test the end point is taken as the time when the mixture first begins to show a fibrin web after gentle periodic rotation. Many times this point is coincidental with complete clot formation. Again it may precede complete clotting by ten to twenty seconds. Violent agitation of the tube after addition of the calcium chloride will delay the clot formation.

During the formation of a clot it has been determined that the pH of the solution is only slightly altered toward the alkaline side. Samples of serum before, during, and after clot formation do not vary appreciably in their pH range. Consequently in most instances it is not necessary to regulate this factor since the normal buffer systems existing in the blood plasma are sufficient to keep the pH within normal limits under conditions of the test.

TABLE IV

TIME GROUP (SECONDS)	CASES	PER CENT
55-60	15	5.8
60-65	45	18.1
65-70	59	23.9
70-75	67	26.8
75-80	58	23.2
80-85	6	2.2
Total	250	100.0



The incubation period is the time interval during which the mixture of thromboplastin and plasma stands before the addition of the calcium chloride. Table III shows the alteration in prothrombin times with varying periods of incubation. The period of incubation which gave the shortest prothrombin time and the most constant results was found to be seven minutes. The decrease in prothrombin time to an optimum at seven minutes' incubation is probably due to the maximum release and formation of thrombin from the plasma. It is probably necessary for a given concentration of thrombin to be formed before it can act as an enzyme in the clotting mechanism. Although it is well known that, given sufficient time, a very small amount of thrombin can convert large quantities of fibrinogen to fibrin, there is apparently an optimum concentration at which it acts. The spread of individual determinations is probably due to the separate rates of release of thrombin or its precursor, prothrombin. Why there is an increase in prothrombin time with longer than seven minutes'

incubation is not easily understood, since thrombin and prothrombin are believed to be very stable. Some light may be thrown on this question by the work of Mertz, Seegers, and Smith,[26] who have suggested that an antiprothrombin is formed which may be thrombin itself.

In all tests involving colloidal chemistry unexplained variations commonly occur. If this fact is not recognized, considerable error may creep into their interpretation. For this reason, it is advisable to evaluate a normal control with each sample of supposed pathologic blood. It is also advisable, unless there is other clinical evidence of a prothrombin alteration, that all abnormal values be checked with repeated specimens of blood before therapy is instituted.

CLINICAL EVALUATION

For purposes of clinical evaluation of this test samples of blood from 250 consecutive patients admitted to the Cottage Hospital were tested. None of these patients had clinical evidence of alteration in their blood prothrombin level. The spread of the entire 250 determinations was from a minimum of 55 seconds to a maximum of 85 seconds. Table IV indicates that the range of normal values for this test lies between 60 and 80 seconds, with an average of 70 seconds since 92 per cent of the cases fall in this bracket. The determinations of the prothrombin time can be converted to per cent of prothrombin by considering the normal or 100 per cent to have a prothrombin time of 70 seconds.

We also used this test as a diagnostic procedure on two patients who were suspected of having lowered prothrombin levels of their blood. An omentopexy was performed on one patient with advanced cirrhosis. During his convalescence this patient developed bleeding from his gums and gastrointestinal tract. Prothrombin determinations were made, and an initial value of 187 seconds was found. Therapy with vitamin K reduced this to 95 to 100 seconds, with a marked cessation in bleeding within a period of twenty-four hours. A second patient had marked jaundice of an obstructive nature and an elevated prothrombin time of nearly 200 seconds. Subsequent treatment with vitamin K reduced this to 85 seconds in the course of three days.

SUMMARY

Due to the objections to the present tests in use for the determination of prothrombin in blood, a modified technique has been evolved which overcomes these defects and in addition has the essential factors of adaptability, simplicity, accuracy, and economy. A new thromboplastic agent which is stable and easily prepared has been made from sheep brain. The modified technique has been adequately tested clinically and has been found to be satisfactory.

REFERENCES

1. Quick, A. J., Stanley-Brown, M., and Bancroft, F. W.: Study of Coagulation Defect in Hemophilia and in Jaundice, Am. J. M. Sc. **190**: 501, 1935.
2. Bancroft, F. W., Kugelmass, I. N., and Stanley-Brown, M.: Evaluation of Blood Clotting Factors in Surgical Diseases, With Special Reference to Thrombosis and Embolism and Certain Bleeding Conditions, Ann. Surg. **90**: 161, 1929.
3. Linton, R. R.: Relation of Blood Fibrin to Hemorrhagic Diathesis of Obstructive Jaundice, Ann. Surg. **96**: 394, 1932.
4. Scott, F. H., and Chamberlain, C.: Calcium and Coagulation of Blood, Proc. Soc. Exper. Biol. & Med. **31**: 1054, 1934.

5. Ferguson, J. H.: Observations on Alterations of Blood Platelets as Factor in Coagulation of Blood, Am. J. Physiol. **108**: 670, 1934.
6. Ravdin, I. S., Riegel, C., and Morrison, J. L.: Coagulation of Blood, Ann. Surg. **91**: 801, 810, 1930.
7. Wangensteen, O. H.: Hemorrhagic Diathesis of Obstructive Jaundice and Its Treatment, Ann. Surg. **88**: 845, 1928.
8. Nygaard, K. K.: Coagulability of Blood Plasma; Remarks on Technique of Its Determination, Proc. Staff Meet., Mayo Clin. **9**: 151, 1934.
9. Howell, W. H., and Cekada, E. B.: Cause of Delayed Clotting of Hemophilic Blood, Am. J. Physiol. **78**: 500, 1926.
10. Bancroft, F. W., Quick, A. J., and Stanley-Brown, M.: Anticoagulants of Blood, Proc. Soc. Exper. Biol. & Med. **32**: 164, 1934.
11. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Plasma Prothrombin Levels in Various Vertebrates, Am. J. Physiol. **125**: 296, 1939.
12. Quick, A. J.: Nature of Bleeding in Jaundice, J. A. M. A. **110**: 1658, 1938.
13. Smith, H. P., Warner, E. D., and Brinkhous, K. M.: Prothrombin Deficiency and Bleeding Tendency in Liver Injury (Chloroform Intoxication), J. Exper. Med. **66**: 801, 1937.
14. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Quantitative Study on Blood Clotting; Prothrombin Fluctuations Under Experimental Conditions, Am. J. Physiol. **114**: 667, 1936.
15. Quick, A. J.: On Action of Heparin and Its Relation to Thromboplastin, Am. J. Physiol. **115**: 317, 1936.
16. Ferguson, J. H.: Quantitative Relationship of Calcium and Cephalin in Experimental Thrombin Formation, Am. J. Physiol. **123**: 341, 1938.
17. Ferguson, J. H.: Blood Calcium and Calcium Factor in Blood Coagulation, Physiol. Rev. **16**: 640, 1936.
18. Quick, A. J.: Coagulation Defects in Sweet Clover Disease and in Hemorrhagic Chick Disease of Dietary Origin; Consideration of Source of Prothrombin, Am. J. Physiol. **118**: 260, 1937.
19. Quick, A. J.: On Various Properties of Thromboplastin (Aqueous Tissue Extracts), Am. J. Physiol. **114**: 282, 1936.
20. Smith, H. P., Warner, E. D., and Brinkhous, K. M.: Lung Extract in Blood Clotting, Am. J. Physiol. **107**: 63, 1934.
21. Snell, A. M.: Symposium on Hemorrhagic Diathesis in Cases of Jaundice: Its Relation to Vitamin K Deficiency; Preliminary Report: Clinical and Experimental Conditions Associated With Deficiency of Prothrombin, Proc. Staff Meet., Mayo Clin. **13**: 65, 1938.
22. Nygaard, K. K.: Coagulability of Blood Plasma, Proc. Staff Meet., Mayo Clin. **7**: 544, 1932.
23. Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P.: Control of Vitamin K Therapy. Compensatory Mechanisms at Low Prothrombin Levels, Proc. Soc. Exper. Biol. & Med. **40**: 595, 1939.
24. Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P.: Blood Coagulation During Infancy, Proc. Soc. Exper. Biol. & Med. **41**: 181, 1939.
25. Quick, A. J., and Grossman, A. M.: Prothrombin Concentration in Newborn, Proc. Soc. Exper. Biol. & Med. **41**: 227, 1939.
26. Mertz, E. T., Seegers, W. H., and Smith, H. P.: Inactivation of Prothrombin by Purified Thrombin Solutions, Proc. Soc. Exper. Biol. & Med. **41**: 657, 1939.

AN INEXPENSIVE ALTERNATING CURRENT PHOTOELECTRIC COLORIMETER*

RUSSELL H. MORGAN, M.D.,† AND SIDNEY WEINHOUSE, PH.D.‡
CHICAGO, ILL.

A RECENT communication[1] described an inexpensive direct-reading photoelectric colorimeter suitable for clinical use and designed to operate from small, portable dry batteries. In some circumstances the use of an instrument operated from alternating current may be more convenient. Alternating current colorimeters that regulate their light source potential by means of so-called constant-voltage transformers, however, have not been entirely satisfactory. Their erratic performance can be traced to line-voltage variations, not completely minimized by the regulating devices that were available. Recently transformers with adequate control have been developed, making possible the modification of our battery colorimeter for use with alternating current without loss of stability or precision. The new model (Fig. 1) can be constructed easily by anyone familiar with electrical apparatus from parts obtainable at a very low cost from any radio or electric supply house.

DESIGN

The colorimeter shown schematically in Fig. 2 operates as a direct-reading instrument. A solution whose concentration is to be determined is subjected to the usual chemical procedure prerequisite to a colorimetric examination. A sample of the resultant preparation is placed in a test tube, and light from a small incandescent lamp is projected through it. The transmitted light, after passing through a selective color filter, falls on the sensitive surface of a photoelectric cell; in response to the radiation a small electric current is generated. The current is amplified many times by a vacuum tube amplifier and is recorded by a milliammeter. This reading is transposed into terms of solution concentration by a graph prepared after calibrating the instrument with samples of known concentration.

The light source, *L. S.*, is a lens-end type, No. 222 Mazda flashlight bulb. This lamp by its unique construction provides abundant light at relatively low filament temperatures, thereby assuring long filament life and freedom from undesirable heating effects.

The selective color filters are of the Wratten gelatin variety and are obtainable from the Eastman Kodak Co. We have found four types to suffice for all colorimetric examinations usually encountered in the clinical laboratory. These are the blue, No. 47, used in blood sugar determinations; the green, No. 58,

*From the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago.
Received for publication, December 8, 1939.
†The John Jay Borland Fellow in Medicine.
‡The Seymour Coman Fellow of the Department of Chemistry, University of Chicago.

used in hemoglobin, nonprotein nitrogen, urea nitrogen, uric acid, and creatinine determinations; the yellow, No. 8, used in calcium and phosphorus determinations; and the red, No. 25, used in cholesterol determinations. The choice of the proper filter for other determinations will be discussed later.

The photoelectric cell, *Ph*, is a rubidium-coated R. C. A. type 926 phototube. Its spectral characteristics make it especially suitable for colorimetry. Although its current response to incident light is extremely small, expensive galvanometers or microammeters are not needed in its circuits because of the tube's adaptability to current amplifier circuits.

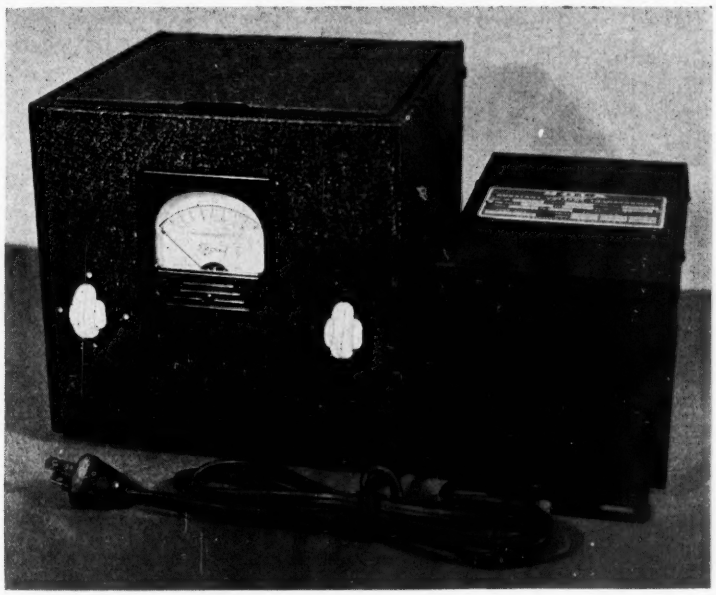


Fig. 1.—The colorimeter. The control on the left changes selective color filters; that on the right operates the power switch and regulates light source intensity.

The amplifier is a single stage, twin triode type. The two triodes of an R. C. A. type 6F8-G radiotron, V_1, are arranged in a balanced Wheatstone bridge circuit in which plate resistances occupy two of the arms; R_4, a 100-ohm potentiometer, and R_b and R_L, 1,000-ohm, 0.5-watt resistors, occupy the remaining two. The balance of the bridge is controlled by R_4. The phototube current flows through R_c, a 20-megohm, 0.5-watt resistor; the potential developed across it is applied directly to the grid of one of the triodes. To maintain the amplifier in a state of balance at all times, R_g, another 20-megohm, 0.5-watt resistor, is connected between the grid of the other triode and ground. Proper grid potentials are obtained by R_a, a 200-ohm, 0.5-watt resistor. A 0-1 milliammeter, *M. A.*, connected across the amplifier bridge, measures the amplified phototube current. A 0.02-microfarad condenser, C_3, inserted across R_c, slows the action of the milliammeter.

The great stability of a twin triode amplifier makes it preferable to other types. Several were set up in the laboratory and operated continuously for 500 hours. All remained balanced during this time, even though line-voltage fluctuations often were as great as 10 per cent. When the power to an amplifier

was turned on after having been shut off for several hours, balance was not disturbed. Once an amplifier is balanced, therefore, subsequent readjustments are unnecessary. Another advantage of the twin triode amplifier is its linear current response. The balanced circuit arrangement makes linearity well within 2 per cent easily possible.

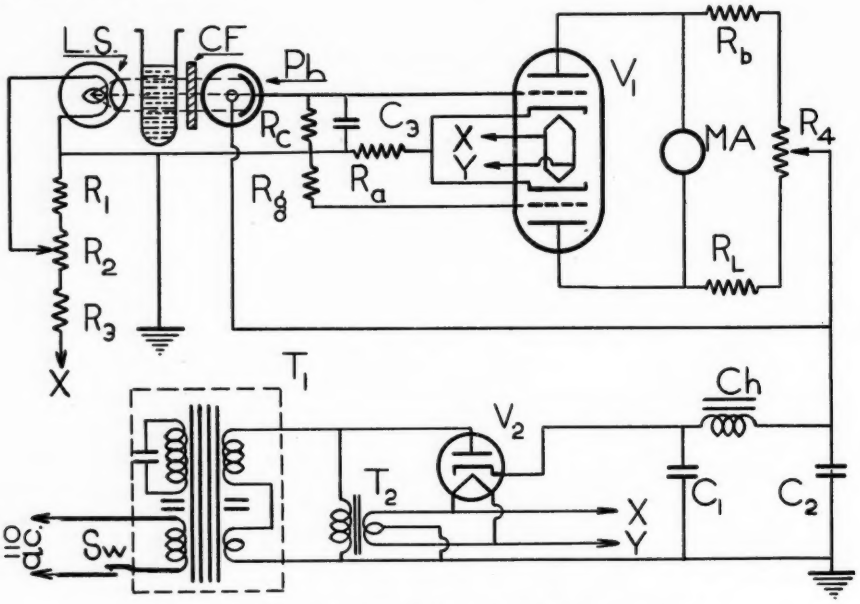


Fig. 2.—Schematic diagram of the colorimeter circuit.

Electric power is obtained from 115-volt, 60-cycle outlets. The necessity of maintaining light source potentials constant within very narrow limits is obvious. Excellently regulated current is provided for the colorimeter by the constant voltage transformer, T_1, a standard stock model, No. 3000, manufactured by the Sola Electric Co. of Chicago. The transformer delivers 30-volt amperes, and the 115-volt output is maintained within plus or minus 1 per cent for a total primary variation of 30 per cent. In contrast to many other transformers, regulation is instantaneous and unaffected by line transients. A simple half-wave rectifier circuit, with an R. C. A. type 1-V radiotron, V_2, and a filter network composed of two 8-microfarad condensers, C_1 and C_2, and a 50-henry, 15 Ma. choke, *Ch*, supplies direct current potentials to the phototube and to the amplifier plate circuit. The center-tapped 6.3-volt, 1.2-ampere transformer, T_2, supplies the filament potentials for the amplifier and rectifier tubes. It also furnishes the filament of the flashlight lamp through the resistor network R_1, a 5-ohm 0.5-watt resistor, R_2, an 8-ohm potentiometer, and R_3, a 20-ohm, 0.5-watt resistor. Intensity of the light source is controlled by R_2. Power is turned on and off by a single pole, single throw switch, *Sw*, attached to the rear of R_2.

CONSTRUCTION

The various components of the colorimeter, with the exception of the constant voltage transformer, T_1, are assembled in a black steel cabinet, 7.5 by 7.5 by 7 inches. The milliammeter, a 3-inch, square case model, and the potenti-

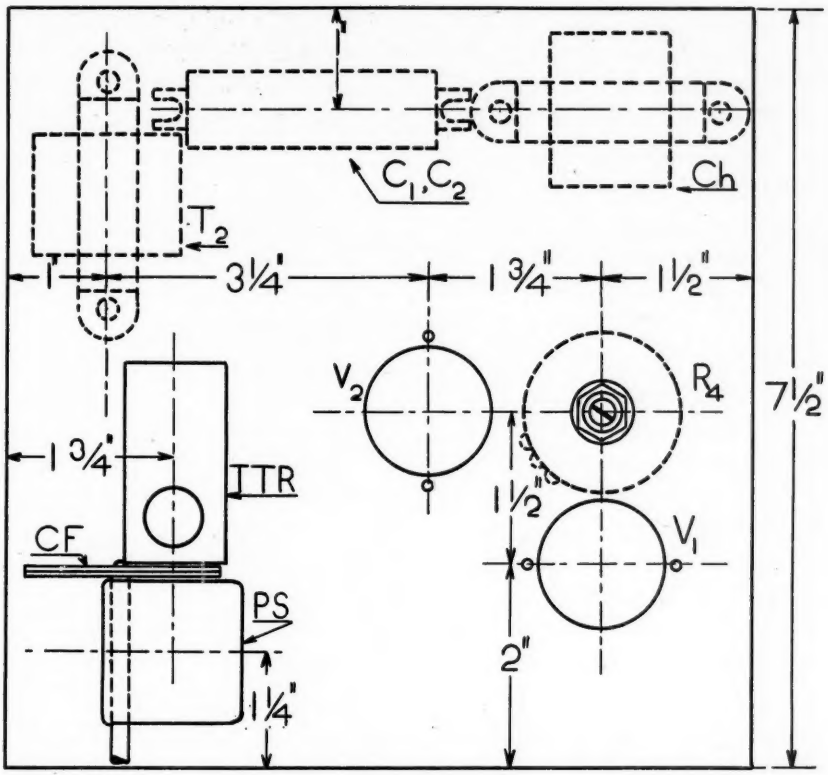


Fig. 3.—The subpanel layout. C_1 and C_2, filter condenser block; *CF*, selective color filter assembly; *Ch*, filter choke; *PS*, phototube shield; R_4, amplifier balancing potentiometer; T_2, 6.3 volt transformer; *TTR*, test tube receptacle; V_1, amplifier tube socket; V_2, rectifier tube socket.

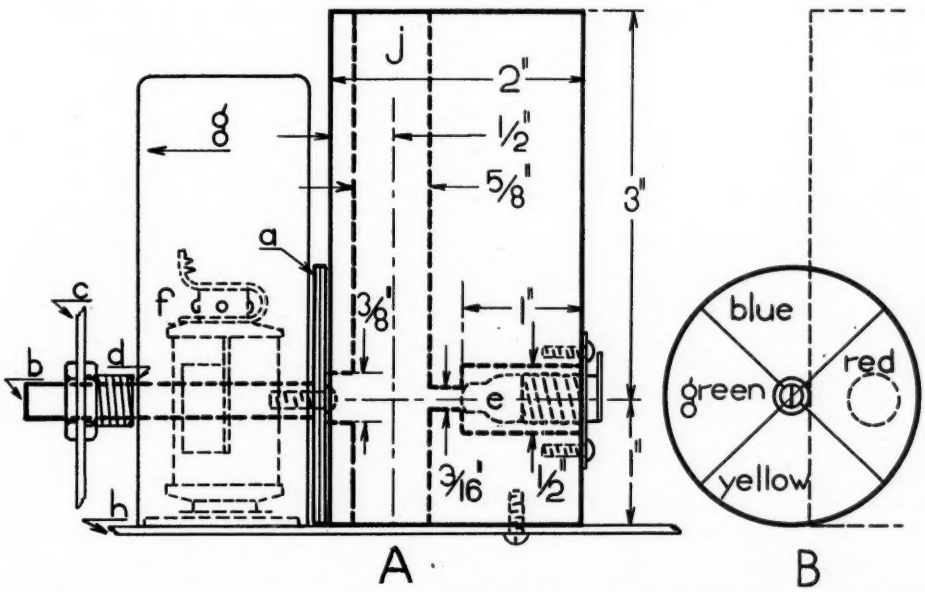


Fig. 4.—*A*, optical system; *a*, circular glass plates with interposed selective color filters; *b*, filter control shaft; *c*, front panel; *d*, shaft bearing; *e*, projection chamber containing flashlight lamp; *f*, phototube and socket; *g*, phototube shield; *h*, subpanel; *j*, test tube receptacle. *B*, arrangement of selective color filters between circular glass plates.

ometer, R_2, are mounted on the front panel; the remaining parts are placed inside the cabinet on a subpanel and arranged as in Fig. 3.

The structural details of the optical system are shown in Fig. 4*A*. An aluminum block, 4 by 2 by 1 inches, drilled according to the specifications outlined, forms a projection chamber for the flashlight lamp and a receptacle in which test tubes containing solutions to be examined can be inserted. The flashlight lamp is mounted in a miniature screw base socket firmly bolted to the block. Light passes from the projection chamber to the test tube receptacle through an opening $\frac{3}{16}$ inch in diameter; another opening, $\frac{3}{8}$ inch in diameter, on the opposite side of the receptacle, conducts transmitted light to the phototube, mounted directly in front of it within a metal shield, $3\frac{1}{2}$ by $1\frac{3}{8}$ by $1\frac{3}{8}$ inches, to exclude stray light.

The four selective color filters are mounted between two circular glass plates, 2 inches in diameter and $\frac{1}{16}$ inch in thickness, having holes $\frac{5}{32}$ inch in diameter drilled through the centers and sealed at the edges with Duco cement (Fig. 4*B*). This filter assembly is placed between the aluminum block and phototube shield and is bolted to a brass shaft, 3 inches long and $\frac{1}{4}$ inch in diameter, supported by a bearing attached to the front panel of the colorimeter. By rotating the shaft the operator can quickly move any of the four filters into the path of the light beam.

OPERATION

Balancing the Bridge.—When the colorimeter has been assembled, the first procedure is to balance the amplifier bridge. The flashlight lamp is removed from its socket, the line cord plugged into an alternating current outlet, and the potentiometer, R_2, rotated to the right. In the first few degrees of rotation, the switch, *Sw*, is closed and the power turned on. Before any adjustments are made, the colorimeter should operate continuously for twenty-four hours. Since new amplifier tubes require several hours of heating to become stabilized, efforts to balance the bridge permanently before stability is attained are wasted. When the prescribed time has elapsed, the potentiometer, R_4, mounted on the subpanel of the instrument, is rotated until the milliammeter needle is brought accurately to its zero scale position. The amplifier circuit is then balanced and needs to be readjusted only if an amplifier tube is changed. When the flashlight lamp is replaced in its socket, the colorimeter is ready for operation.

Matching the Absorption Tubes.—Ordinary soft glass test tubes, 5 or 6 inches long and $\frac{5}{8}$ inch in outside diameter, are used as absorption cells. To obtain a set of tubes of identical light transmission, it is suggested that several dozen be purchased, cleaned carefully with sulfuric acid-dichromate cleaning mixture, and filled with distilled water. A sample tube is placed in the instrument, the power turned on, and the potentiometer, R_2, rotated until the milliammeter needle is brought to any convenient position near full scale. The meter deflection produced by each of the tubes is noted, and those whose readings deviate more than 0.5 of 1 per cent from the mean are discarded. The remaining ones are kept for colorimetric use.

Choosing a Selective Color Filter.—Photoelectric colorimeters measure the percentage light absorption of solutions submitted for examination. The re-

lationship between light absorption and solution concentration can be expressed, according to the Beer-Lambert law, by the equation,

$$\log \text{ per cent absorption} = KCX,$$

where C is the concentration of the solution examined, X is the length of the solution through which light passes, and K is a constant. For a particular set of matched absorption tubes, X is a constant; therefore, the concentration is proportional to the logarithm of the light absorbed or inversely proportional to the logarithm of the light transmitted. The value of K depends upon the wave length of the light transmitted by the solution. In some portions of the spectrum the absorption is relatively small; in regions corresponding to the positions of the solution's characteristic absorption bands it is much greater. When various selective color filters that transmit light only in limited regions of the spectrum are used, the absorption can be made to have a wide range of values. It obviously is desirable not to have the absorption too small, for then it becomes difficult to measure. On the other hand, it is evident by differentiating the equation that, when the absorption becomes too great, large changes in concentration produce very small changes in light absorption, equally difficult to estimate. Satisfactory performance is attained if the absorption is restricted to less than 70 per cent of the incident light. Generally, a filter of the color complementary to that of the solution examined fulfills these conditions. In some instances, however, as for example in the Folin-Wu determination of blood sugar, the color density of the solution is so great that a filter having a color similar to that of the solution should be used. Regulation can be achieved in other ways, namely, by altering the amount of substance taken for analysis or by changing the volume of the final dilution.

When the proper filter is to be selected, a solution is prepared having the maximum concentration desired to be determined. With a test tube of distilled water in the receptacle, the meter is set at full scale deflection by the potentiometer, R_2. Then the distilled water is replaced by the colored solution, and the readings are noted with each of the four filters. That solution which produces a deflection not exceeding about 70 per cent of the full scale is chosen for the particular determination.

Making a Determination.—A series of standard solutions having concentrations ranging from the lowest to the highest values expected to be encountered in the laboratory is prepared. A solution having zero concentration is also included. With the proper filter in place and a tube of distilled water in the receptacle, the meter is set at full scale deflection. The distilled water is then replaced in turn by each of the standard solutions and the meter reading of each recorded.

Calibration of the instrument can be carried out in several different ways. One method is to determine the specific extinction coefficient, $E_{sp.}$, in the equation,

$$E_{sp.} = \frac{\log \frac{T_o}{T}}{C},$$

where T_o is the meter reading of the solution having zero concentration, T is the meter reading of one of the standard solutions, and C is its concentration. The mean of the various values of the coefficient can then be used to calculate the concentration of any solution according to the formula,

$$C = \frac{\log \frac{T_o}{T}}{E_{sp.}}.$$

A more direct method of calibrating the colorimeter is simply to make a graph plotting solution concentrations against the logarithms of the meter readings. A line is drawn through the points, and concentrations can be read directly from the graph. An improvement of this method is to plot the graph on semilogarithmic paper, with meter readings placed along the logarithmic axis. Observations then can be converted directly to concentration values without calculation. Once the calibration curve is made, it is unnecessary to prepare standard solutions in making subsequent determinations, except as an occasional check or when new reagents are prepared. When a determination is made, the power to the instrument is simply turned on; two minutes are allowed for the amplifier and flashlight lamp to heat; the proper selective color filter is inserted; the potentiometer, R_2, is adjusted for a full scale deflection of the meter with a test tube of distilled water in its receptacle; and the reading of the specimen is observed. The concentration is evaluated from the calibration curve.

If a series of consecutive determinations is to be made, it is advisable from time to time to check the position of R_2. Fatigue of the light source causes a slow drift of the meter needle, making slight readjustments necessary. When the colorimeter has been in operation for ten minutes, the rate of drift is 0.25 of 1 per cent per minute.

In addition to the general principles of operation outlined, no attempt will be made to discuss the application of the instrument to specific problems. Many recently published reports describe the use of direct-reading photoelectric colorimeters in a great many clinical laboratory procedures.

SUMMARY

An inexpensive photoelectric colorimeter suitable for clinical use and designed to operate from alternating current outlets is described. Complete details of design, construction, and operation are included. Performance comparing favorably with that of commercial colorimeters can be expected. Operation is extremely simple.

REFERENCE

1. Morgan, R. H., and Weinhouse, S.: An Inexpensive Portable Photoelectric Colorimeter, Am. J. Clin. Path. 10: 114, 1940.

THE PHOTELOMETRIC DETERMINATION OF BILIRUBIN IN URINE WITH DIAZOBENZENESULFONIC ACID*

WILLIAM H. GOODSON, JR., M.D., AND CHARLES SHEARD, PH.D.
ROCHESTER, MINN.

A SIMPLE, rapid and accurate procedure for the quantitative estimation of urinary bilirubin has been developed for use with the Sheard-Sanford photelometer. This instrument makes use of spectral filters selected to transmit light chiefly in the region of maximal absorption of the substance under test, and thereby deletes the effects of extraneous colored substances or other regions of spectral absorption which may be present in the solution. The photelometer also eliminates personal error in color matching and dispenses with the need for artificial standards.

The photelometer is calibrated for bilirubin in the manner described by Osterberg, except that it is not necessary to evaporate the solution of bilirubin in chloroform to dryness. A weighed sample of bilirubin is dissolved in chloroform, and serial dilutions corresponding to convenient concentrations of bilirubin are prepared. Two milliliter portions of these solutions, to each of which is added a small quantity of alcohol, are treated with 1 ml. of the diazo reagent and are made up with alcohol to a final volume of 10 ml. These solutions are compared with water in the photelometer.

For the calibration in terms of bilirubin, a spectral filter that has its maximal transmission in the region of 530 to 550 mμ is chosen. It is most satisfactory to construct the calibration curve on semilogarithmic paper; this makes it easy to select any desired scale value (such as 50) as the reading which corresponds to a solution free of bilirubin. Less than full scale deflection may be desired when small amounts of solution are to be examined; for, if light passes above the meniscus of the solution, the accuracy of the readings is lost.

METHOD

A method for the determination of urinary bilirubin is herein proposed.

Solutions.—1. *Caffeine sodio-benzoate and buffer solution.* Mix equal parts of a 25 per cent solution of caffeine sodio-benzoate† in distilled water and Sörensen's phosphate buffer pH 6.6. The buffer is most conveniently prepared‡ by mixing 60 ml. of one-fifth molar potassium dihydrogen phosphate (13.617 Gm. of KH_2PO_4 in 500 ml. of water), 40 ml. of one-fifth molar dibasic sodium phosphate (17.814 Gm. of $Na_2HPO_4 \cdot 2H_2O$ in 500 ml. of water), and 200 ml. of water.

*From the Mayo Clinic, Rochester.
Received for publication, March 25, 1940.

†Caffeine sodio-benzoate (U. S. P. X) is defined as "a mixture of caffeine and sodium benzoate which contains, when dried to a constant weight at 80° C., not less than 47 per cent nor more than 50 per cent of anhydrous caffeine ($C_8H_{10}O_2N_4$), and not less than 50 per cent nor more than 53 per cent of sodium benzoate ($NaC_7H_5O_2$)."

‡Modified from the description of Clark.

2. *Acid blank solution.* Dilute 7.5 ml. of concentrated hydrochloric acid with water to make one liter.

3. *Diazo reagent.* Note that although this diazo reagent contains the same substances as Ehrlich's diazo reagent and many of its modifications (such as the so-called van den Bergh reagent), the concentrations are less in the one described here.

I. Sulfanilic acid ($C_6H_4(NH_2)(SO_3H) + 2H_2O$) 1 Gm., concentrated hydrochloric acid 15 ml., and water to make one liter.

II. Sodium nitrite ($NaNO_2$) 0.5 Gm. and water to make 100 ml. For use, take 0.3 ml. of solution II to 10 ml. of solution I, and dilute with water to make 20 ml.

PROCEDURE

To each of two tubes, "blank" and "test," add 2 ml. of fresh centrifuged urine and 2 ml. of caffeine sodio-benzoate and buffer solution (1). Mix well by rotating the tubes, and let stand for ten minutes or a little longer. To tube "blank" add 1 ml. of acid blank solution (2) and 5 ml. of water. To tube "test" add 1 ml. of diazo reagent (3) and 5 ml. of water.

Let both tubes stand for ten to fifteen minutes. With the same filter which was used in calibrating the photelometer in place and the contents of the "blank" tube in the test cell, adjust the instrument to the scale value which corresponds to a solution free of bilirubin, and then substitute the contents of tube "test" in another cell. From the scale reading and the calibration curve, determine the content of bilirubin. If the content of bilirubin in the urine is too high to be read within the range of the instrument, the urine may be diluted with water, and the test repeated.

SPECTROPHOTOMETRIC AND PHOTELOMETRIC EXPERIMENTS

With methods hitherto employed for the quantitative determination of urinary bilirubin, it has been necessary to resort to adsorption of the bilirubin to an inert precipitate (Hunter,[8] Naumann, Jendrassik, and Grof) in order to avoid interference from other urinary substances. In the case of the procedure which we have described, this same result is achieved through control of the pH of the reaction mixture and use of the urine (a colored solution), properly diluted, as a "blank" for adjusting the photelometer before taking a reading. To demonstrate the validity of this procedure, we have made certain spectrophotometric studies of solutions of bilirubin, urine, and bilirubin in urine.

If a sample of urine from a jaundiced patient is treated with caffeine sodio-benzoate, diazobenzenesulfonic acid in hydrochloric acid solution,* and is buffered to a final pH of 4 to 5.5., a definite red tint appears. The spectrophotometric comparison of such a solution with water in terms of percentage of light transmitted at various wave lengths is shown in Fig. 1 (curve *a*). A similar comparison of the same urine treated with caffeine sodio-benzoate, buffer, and an amount of hydrochloric acid equivalent to that in the diazo reagent (that is, the "blank" solution already described) is shown in Fig. 1 (curve *b*). The sample to which diazo reagent has been added transmits much less light in the

*Hereinafter designated the "diazo reagent."

region between 500 and 600 mμ than does the untreated sample. In the region between 450 and 520 mμ, however, various urinary pigments cause a considerable reduction in the light transmitted by the untreated sample. An allowance for this absorption, common to the two samples, is made by comparing the sample to which the diazo reagent has been added with the sample untreated (except for identical acidification, buffering and dilution), instead of with water. The resulting percentage transmission of light at various wave lengths is shown in Fig. 1 (curve *c*); there has appeared, as the result of the addition of the diazo reagent, a distinct zone of increased spectral absorption with a maximal value between 520 and 540 mμ. The spectrophotometric measurement of the light transmitted by a solution of purified bilirubin treated with the diazo reagent in a mixture of chloroform, alcohol, and water is represented in Fig. 1 (curve *d*) and is essentially identical in character and spectral region of maximal absorption with that represented in Fig. 1 (curve *c*).

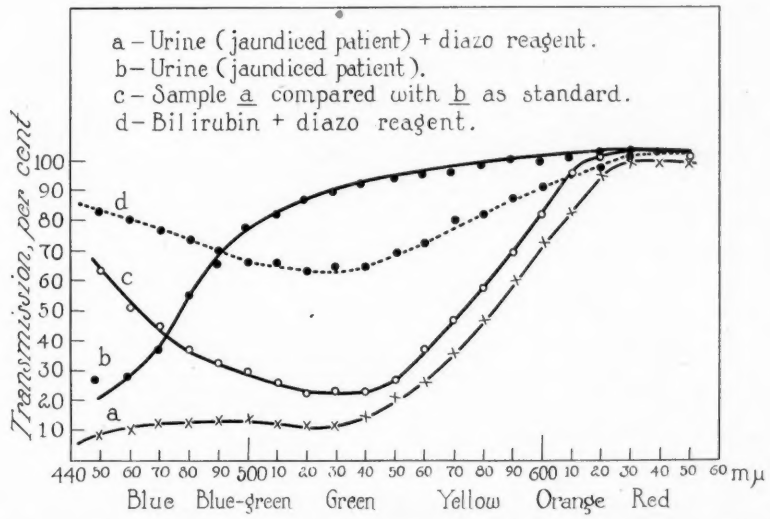


Fig. 1.—Spectrophotometric transmission curves; *a*, of urine (diluted 1:4) from a jaundiced patient after the addition of acetate buffer, diazo reagent, and water, compared with water as a standard; *b*, of urine (diluted 1:4) from the same sample after the addition of acetate buffer, acid blank solution, and water, compared with water as a standard; *c*, of the solution represented by curve *a* when compared with the solution represented by curve *b* instead of water as a standard; *d*, of pure bilirubin dissolved in chloroform, diluted with alcohol, treated with the diazo reagent, and compared with water.

In Fig. 2 (curve *a*) is shown the spectrophotometric curve of light transmitted by a mixture of phosphate buffer, alcohol, diazo reagent, and bilirubin dissolved in sodium carbonate solution (compared with water). A portion of the same sample of bilirubin dissolved in sodium carbonate solution was added to normal urine. A portion of the resulting mixture was treated with caffeine sodio-benzoate, buffer, and the diazo reagent, and was compared spectrophotometrically with another portion of the mixture treated with caffeine sodio-benzoate, buffer, and hydrochloric acid only. Fig. 2 (curve *b*) shows the percentage transmission of light then obtained at various wave lengths. Both the spectrophotometric curves obtained for bilirubin in chloroform and carbonate solutions after treatment with the diazo reagent, and the curve obtained after adding the same re-

agent to bilirubin in urine (using the untreated urine as a standard) are of the same general character and exhibit the same zone of absorption, with maximal absorption at 520 to 540 mμ.

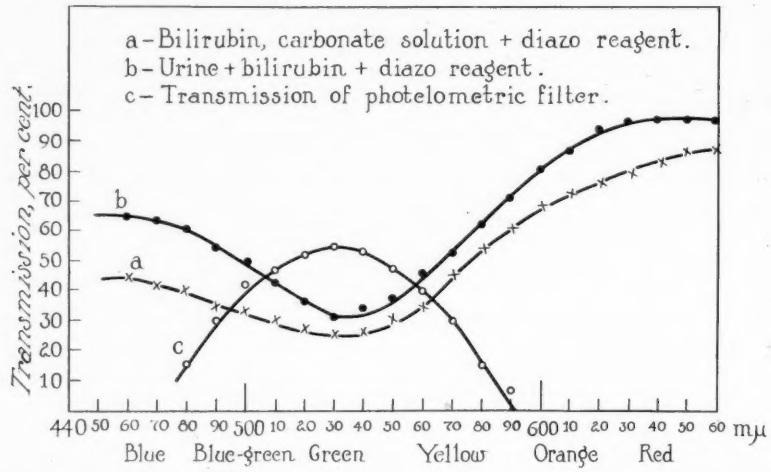


Fig. 2.—Spectrophotometric transmission curves; *a*, obtained when bilirubin dissolved in a solution of sodium carbonate is treated with the diazo reagent in the presence of phosphate buffer (pH 6.6) and alcohol, and compared with water as a standard; *b*, obtained when a mixture of normal urine and bilirubin dissolved in a solution of sodium carbonate is treated with caffeine sodio-benzoate, phosphate buffer, and diazo reagent, and compared with the same mixture treated with caffeine sodio-benzoate, phosphate buffer, and acid blank solution as a standard. Curve *c* represents the percentage of incident light transmitted at various wave lengths by the photelometer filter used in these experiments.

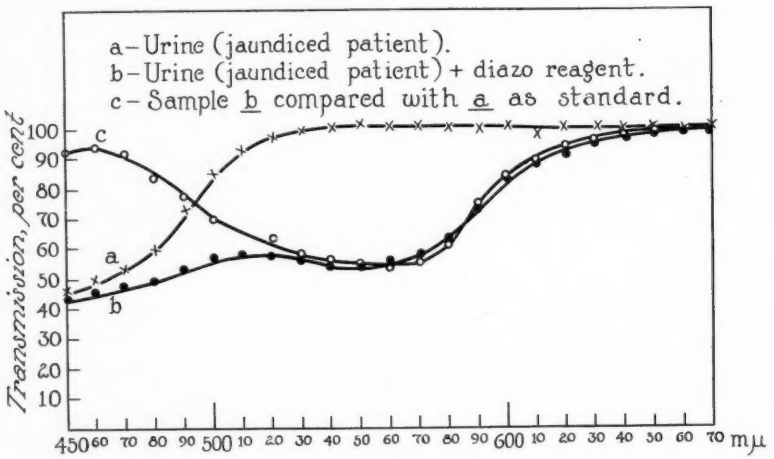


Fig. 3.—Spectrophotometric transmission curves; *a*, of urine from a jaundiced patient, treated with caffeine sodio-benzoate, phosphate buffer, and acid blank solution, as described in the text, and compared with water as a standard; *b*, of a sample of the same urine treated with caffeine sodio-benzoate, phosphate buffer, and diazo reagent, as described in the text, and compared with water as a standard; *c*, of the solution represented by curve *b* when compared with the solution represented by curve *a*, instead of water, as a standard.

Oxyhemoglobin has bands of spectral absorption with their maximal values at 542 and 578 mμ, respectively, and, therefore, it might be presumed that blood would interfere with the photelometric determination of bilirubin. A specimen of urine from a jaundiced patient was divided into two portions: to the first

*Intact erythrocytes, of course, would be removed by centrifugation, along with other debris, from any sample ordinarily submitted to the test.

was added a quantity of hemolyzed blood,* and to the second an exactly equal amount of distilled water. The curves of Figs. 3 and 4 show how the use of the untreated urine blank compensates for the presence of added hemoglobin. In Fig. 3 curve *a* represents the spectrophotometric transmission of the portion diluted with water, prepared as for the "blank" solution, and compared with water; curve *b* contains the data on the same sample after treatment with the diazo reagent as already described; and curve *c* shows the resultant percentage transmission of light when the treated urine is compared with the untreated urine as a standard. In Fig. 4 curve *a* represents the spectrophotometric transmission

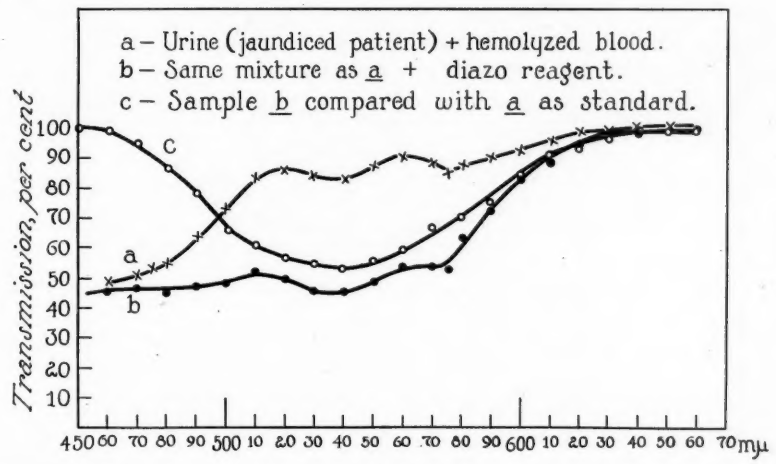


Fig. 4.—Spectrophotometric transmission curves; *a*, of urine from a jaundiced patient (the same sample as used for the curves of Fig. 3), with added hemolyzed blood, treated with caffeine sodio-benzoate, phosphate buffer, and acid blank solution, and compared with water as a standard; *b*, of the same urine-hemolyzed blood mixture treated with caffeine sodio-benzoate, phosphate buffer, and diazo reagent, and compared with water as a standard; *c*, of the solution represented by curve *b* when compared with the solution represented by curve *a* as a standard, instead of with water.

for the portion to which the hemolyzed blood was added and demonstrates the typical absorption curve of oxyhemoglobin superimposed on that of the urine alone; curve *b* represents the spectrophotometric transmission of the urine-hemoglobin mixture after the addition of the diazo reagent (compared with water); and curve *c* represents the spectrophotometric transmission of the urine-hemoglobin-diazo mixture compared with the untreated urine-hemoglobin mixture. It is obvious that curve *c* in Fig. 4 is essentially identical with curve *c* in Fig. 3.

Specimens of urine from several jaundiced patients were divided and treated as just described, and were then assayed for bilirubin in the photelometer. The data are presented in Table I; they clearly demonstrate that the added hemolyzed blood does not impair the accuracy of our method.

We have shown by the foregoing experiments (a) that the development of typical diazobilirubin is not hindered by urinary constituents, and (b) that under the conditions which we have specified, diazobenzenesulfonic acid does not couple with urinary constituents other than bilirubin to produce a colored substance having a zone of absorption in the region between 500 and 600 mμ. We have also shown that pre-existing urinary pigments which produce some absorp-

tion in the spectral region with which we are concerned (that is, 520 to 540 mμ) are entirely compensated for by the use of the diluted acidified urine, untreated with the diazo reagent, as a standard for spectrophotometric or photelometric comparison.

TABLE I

EFFECT OF ADDING HEMOLYZED BLOOD TO URINE

NUMBER	MILLIGRAMS OF BILIRUBIN PER 100 ML. OF	
	URINE	URINE + HEMOLYZED BLOOD
1	3.20	3.20
2	1.30	1.35
3	0.70	0.70
4	1.03	1.03
5	1.70	1.65

When spectrophotometric determinations are made of the transmissions of normal urine before (Fig. 5, curve *a*) and after (Fig. 5, curve *b*) treatment with the diazo reagent, both identically diluted and compared with water, it is again apparent, as in the case of urine from a jaundiced patient (Fig. 1, curves *a* and *b*) that there is a greater absorption of light in the region of the shorter wave lengths after the addition of the diazo reagent. When the sample to which the diazo reagent has been added is compared spectrophotometrically with the untreated sample, however, no characteristic band of absorption appears (Fig. 5, curve *c*). This is probably due to an overshadowing effect of the unchanged urinary pigments. Since the photelometer deletes the effect of the increased absorption which appears to be produced in the deep blue region, this may be disregarded. We know of no other naturally occurring substance in the urine which could react with the diazo reagent under these circumstances to develop the color characteristic of diazobilirubin. Because the transmission curves for the normal urine coupled with the diazo reagent are themselves inconclusive, and because with larger amounts of bilirubin the characteristic absorption band of diazobilirubin develops, we are justified in the conclusion that the maximal absorption of light in the region of 520 to 540 mμ which occurs in urine treated with diazobenzenesulfonic acid as already described is due to bilirubin.

The importance of maintaining an acid reaction when assaying urine for bilirubin as we have described follows from a consideration of the coupling reactions known to occur with diazobenzenesulfonic acid in sodium carbonate solution. This reaction was introduced by Pauly, and together with its modifications has been extensively applied by a number of investigators (Clifford,[3] Hanke and Koessler,[10] Hunter,[6, 7] Nakayama,[13] Hewitt, and others) to many substances, notably the phenols, imidazoles, and certain amino acids. Several of these are included among the seventy possible urinary constituents described by Thierfelder. A few of them, biliverdin, urobilin (Ehrlich), xanthine, hypoxanthine, guanine (Hunter[6]), phenol, and histamine have been specifically tested with the diazobenzenesulfonic acid reagent in acid solution and have been found to produce no color which could interfere with the test for bilirubin. Hunter[7] stated that aromatic amines (of which there are none in Thierfelder's list) will react

with diazobenzenesulfonic acid in acid solution, but he added that "there has never been any question of its specificity in biological fluids for bilirubin." We feel that this conclusion is strongly confirmed by the spectrophotometric curves which we have presented.

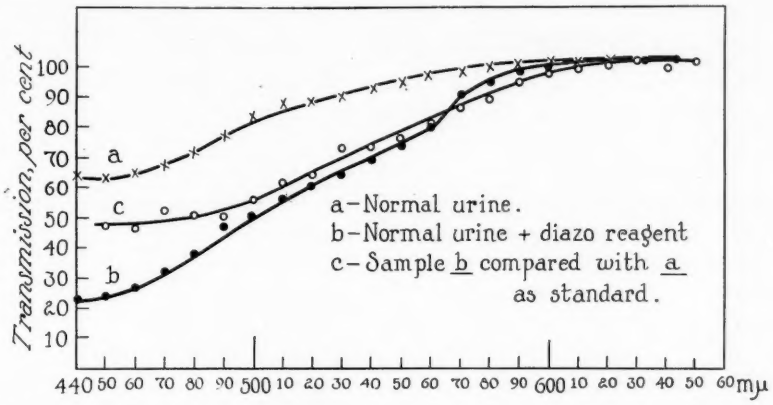


Fig. 5.—Spectrophotometric transmission curves; *a*, of normal urine treated with caffeine sodio-benzoate, phosphate buffer, and acid blank solution, and compared with water as a standard; *b*, of the same sample treated with caffeine sodio-benzoate, phosphate buffer, and the diazo reagent, and compared with water as a standard; *c*, of the solution represented by curve *b* compared with the solution represented by curve *a* as a standard, instead of water.

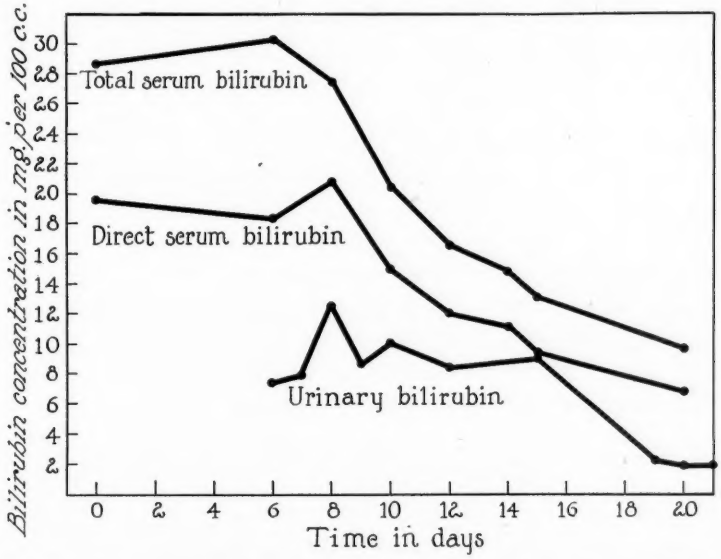


Fig. 6.—Curves representing postoperative fall in total serum bilirubin and urinary bilirubin, expressed in milligrams per 100 ml., over a period of time measured in days. The data were obtained from a case of obstructive jaundice due to carcinoma of the head of the pancreas. The serum bilirubin values were determined by the method of Malloy and Evelyn.

The "Ehrlich diazo reaction," as described in current texts on clinical pathology (for instance, Todd and Sanford), is made in ammoniacal solution. Just what substance produces the test is not definitely known, although it has been studied by Hunter,[7] Sachs and Schmidinger, Miyazaki, and Nakayama. What we have said about the Pauly reaction applies equally well to the "Ehrlich diazo reaction."

The phosphate buffer which we have included in our reaction mixture serves chiefly to prevent the coagulation of albumin by the acid diazo reagent and to maintain the final pH in the range (4 to 5.5) most favorable for the characteristic red color of diazobilirubin.

Although alcohol is generally relied on to insure complete coupling of the diazo reagent with bilirubin in blood serum, it is unsatisfactory for this purpose in urine, because hazy solutions usually result, even though there be no precipitation of albumin, and in many instances the development of color is inhibited.

TABLE II

ACTION OF ALCOHOL ON THE DEVELOPMENT OF DIAZOBILIRUBIN COLOR IN URINE
(Values expressed in milligrams per 100 ml. of urine)

METHOD	NUMBER	DILUTION	
		WITH ALCOHOL	WITH WATER
I	1	0.27	0.47
	2	0.29	0.43
	3	0.15	0.33
	4	0.35	0.12
	5	0.51	0.53
	6	0.51	0.53
	7	0.13	0.63
	8	0.24	0.37
	9	0.31	0.45
	10	0.08	0.15
II	11	0.03	0.07
	11a	0.15	0.38
	11b*	0.08	0.43
	12	0.31	0.49
	13	0.43	0.33
III	14	0.43	0.78
	15	0.37	0.43
	16	0.40	0.47
	17	0.26	0.38
	18	0.36	0.48

Method I: 5 ml. of methanol or water plus 1 ml. of diazo reagent (or acid blank solution), plus 4 ml. of urine; centrifuge.

Method II: same as Method I, except that the urine was acidified with hydrochloric acid to pH 5.5 to 6 before adding the test solutions.

Method III: 2 ml. of centrifuged urine, plus 2 ml. of phosphate buffer pH 6.6; plus 1 ml. of diazo reagent (or acid blank solution), plus 5 ml. of water or methanol.

*Buffered to pH 6.6 with phosphate buffer before the test.

The generally higher values of the aqueous solutions shown in Table II probably reflect the greater accelerating action of urea, which Adler and Strauss discovered in an effort to determine the nature of the "direct" and "indirect" van den Bergh reactions in blood serum. They found that caffeine sodio-benzoate, urea, and certain other diuretic substances greatly accelerated the development of a maximal degree of color by the diazobenzenesulfonic acid coupling reaction with bilirubin. Caffeine sodio-benzoate in 25 per cent solution can be added to urine without the formation of a precipitate and, as shown in Table III, frequently aids in the development of the maximal depth of color and in the prevention of haziness. Fig. 2 (curve *b*) shows that this color is the same as the color developed in other solutions which contain bilirubin. Because of the inevitable presence of urea in urine, the terms "direct" and "indirect" can have no significance as applied to urinary bilirubin.

TABLE III

EFFECT OF CAFFEINE SODIO-BENZOATE ON THE DEVELOPMENT OF DIAZOBILIRUBIN COLOR IN URINE
(Values expressed in milligrams of bilirubin per 100 ml. of urine.)

NUMBER	WITHOUT CAFFEINE	WITH CAFFEINE
19	0.30	0.55
20	0.30	0.40
21	0.99	0.70
22	0.66	0.70
23	1.57	1.57
24	1.25	1.35
25	0.78	0.93
26	Hazy	3.90

Solutions without caffeine were treated as follows: 2 ml. of urine, 2 ml. of phosphate buffer, 1 ml. of diazo reagent (or acid blank solution) and 5 ml. of water. Solutions with caffeine were treated as described in the text.

TABLE IV

QUANTITATIVE RECOVERY OF BILIRUBIN ADDED TO URINE

SAMPLE	MILLIGRAMS OF BILIRUBIN PER 100 ML. OF				PERCENTAGE RECOVERED*
	URINE	BILIRUBIN SOLUTION	URINE + BILIRUBIN		
			ANTICIPATED	FOUND	
1	0.58	1.08	1.66	1.55	93.5
2	0.55	1.17	1.72	1.65	96.0
3	0.47	1.06	1.53	1.45	94.8
4	0.51	1.09	1.60	1.75	109.4
5	0.37	1.09	1.46	1.50	102.7
6	0.40	0.90	1.30	1.25	96.2
7	0.45	0.90	1.35	1.15†	85.1
8	0.23	0.92		1.15	100.0
9	0.53	9.30	9.83	9.30	94.6
10	0.60	3.10	3.70	3.78	102.1

*Average recovery 97.4 per cent; range 85.1 to 109.4 per cent.
†This solution became faintly hazy on the addition of the bilirubin to the urine.

The final test of any analytical procedure is the accuracy of its quantitative recovery of the test substance. The application of this inquiry to bilirubin in urine is vastly complicated by the virtual insolubility of bilirubin except in highly alkaline mixtures or in organic substances which are immiscible with water. After many experments it was found that bilirubin dissolved in a solution of urea and sodium carbonate, a mixture* prepared and furnished through the generosity of Dr. David Klein, Director of the Wilson Laboratories, could be added to urine in most instances without precipitation and without making the solution sufficiently alkaline to invalidate the test. The procedure was as follows: 1 ml. of the diluted bilirubin solution was placed in each of two 10 ml. volumetric flasks, and was made up in the one instance with urine and in the other with distilled water to the volume of 10 ml. In a third 10 ml. volumetric flask were placed 1 ml. of distilled water and 9 ml. of urine. From each of these mixtures, 2 ml. were then taken for testing according to the method that we have described, the diluted urine being used as a "blank" for the urine alone and the urine plus bilirubin, and distilled water as the "blank" for the bilirubin

*The original mixture contained in each milliliter 22 mg. of bilirubin, 300 mg. of urea, and 10 mg. of sodium carbonate. This solution is made up at the rate of 300 mg. of urea and 22 mg. of bilirubin per 100 ml. The average daily excretion of urea in normal urine is such that a concentration of at least 1,000 mg. per 100 ml. is maintained. It may be that even though the urine is acid, the bilirubin excreted by the kidneys is held in solution in some way by the urea present.

alone. On the average, as shown by the results in Table IV, 97.4 per cent of the added bilirubin was recovered.

The test here described was developed in connection with certain experiments on the excretion of bilirubin in which a quantitation of urinary bilirubin seemed desirable. In Fig. 6 are shown the relative concentrations of serum bilirubin and urinary bilirubin from a patient whose obstructive jaundice was subsiding postoperatively.

SUMMARY

On the addition of an acid solution of diazobenzenesulfonic acid to urine that contains bilirubin, the typical color of diazobilirubin is developed. The use of a suitable phosphate buffer prevents the precipitation of albumin and maintains the pH of the reaction mixture in the zone between 4 and 5.5. Caffeine sodio-benzoate may be added to assure the development of the maximal depth of color and to aid in the prevention of haziness. It is emphasized that the Pauly reaction and the "Ehrlich diazo reaction" take place in alkaline solution.

Spectrophotometric curves show that the red color developed in urine on the addition of caffeine sodio-benzoate, phosphate buffer, and acid solution of diazobenzenesulfonic acid can be used to estimate bilirubin in the photelometer, provided the instrument is standardized against a similarly diluted sample of the untreated urine.

REFERENCES

1. Adler, Erich, and Strauss, Leo: Beitrag zum Mechanismus der Bilirubinreaktion im Blut, Klin. Wchnschr. **1**: 2285, 1922.
2. Clark, W. M.: The Determination of Hydrogen Ions. An Elementary Treatise on Electrode, Indicator and Supplementary Methods; With an Indexed Bibliography on Applications, ed. 3, Baltimore, 1928, Williams & Wilkins Co., 715 pp.
3. Clifford, Winifred M.: A Method for the Colorimetric Estimation of Carnosine, Biochem. J. **15**: 400, 1921.
4. Ehrlich, P.: Sulfodiazobenzol, ein Reagens auf Bilirubin, Centralbl. f. klin. Med. **4**: 721, 1883.
5. Hewitt, L. F.: The Diazo Reaction in Uraemic Sera, Biochem. J. **19**: 171, 1925.
6. Hunter, George: Estimation of Carnosine in Muscle Extract—A Critical Study, Biochem. J. **16**: 640, 1922.
7. Hunter, George: The Diazo Reaction in Urine, Biochem. J. **19**: 25, 1925.
8. Hunter, George: A Diazo Method for Detecting Bilirubin in Urine, Canad. M. A. J. **23**: 823, 1930.
9. Jendrassik, L., and Grof, P.: Verfahren zur photometrischen Bestimmung des Bilirubins im Harn, Biochem. Ztschr. **296**: 71, 1938.
10. Koessler, K. K., and Hanke, M. T.: Studies on Proteinogenous Amines. II. A Microchemical Colorimetric Method for Estimating Imidazole Derivatives, J. Biol. Chem. **39**: 497, 1919; VII. The Quantitative Colorimetric Estimation of Histidine in Protein and Protein-Containing Matter **43**: 527, 1920; VIII. A Method for the Quantitative Colorimetric Estimation of Histamine in Protein and Protein-Containing Matter **43**: 543, 1920; XIV. A Microchemical Colorimetric Method for Estimating Tyrosine, Tyramine, and Other Phenols, **50**: 235, 1922.
11. Malloy, H. H., and Evelyn, K. A.: The Determination of Bilirubin With the Photoelectric Colorimeter, J. Biol. Chem. **119**: 481, 1937.
12. Miyazaki, Misaki: Studies on the Diazo-Body in Typhoid Urine, J. Biochem. **18**: 379, 1933 (Abstr.); The Diazo Compound in Typhoid Urine, Chem. Abstr. **28**: 819, 1934.
13. Nakayama, Sadajiro: Zur Kenntnis des Diazoharns: III. Über die chemische Zusammensetzung des Diazoharns bei Scharlach, J. Biochem. **19**: 33, 1934 (Abstr.); Diazo Urines. III. Chemical Composition of Diazo Urines in Scarlatina, Chem. Abstr. **28**: 2405, 1934; IV. Über die chemische Zusammensetzung des Diazoharns bei Masern, J. Biochem. **19**: 39, 1934 (Abstr.); IV. Chemical Composition of Diazo Urines in Measles, Chem. Abstr. **28**: 2405, 1934; V. Über die Diazoreaktion des Pockenharns, J. Biochem. **21**: 439, 1935 (Abstr.); V. Diazo Reaction of the Urine From Smallpox, Chem. Abstr. **29**: 8123, 1935; VI. Zur Kenntnis der Antoxypro-

teinsäure, J. Biochem. **21**: 443, 1935 (Abstr.); VI. Studies on Antoxyproteic Acid, Chem. Abstr. **29**: 8123, 1935.
14. Naumann, H. N.: A New Test for Bilirubin in the Urine and Its Use for the Detection of Bilirubin in Normal Urine, Biochem. J. **30**: 762, 1936.
15. Osterberg, A. E.: The New van den Bergh Reaction for the Determination of Serum Bilirubin Utilizing the Photelometer, J. LAB. & CLIN. MED. **22**: 729, 1937.
16. Pauly, H.: Über die Konstitution des Histidins, Ztschr. f. physiol. Chem. **42**: 508, 1904.
17. Pharmacopeia of the United States of America, Tenth Decennial Revision, Philadelphia, 1925, J. B. Lippincott Co., 626 pp.
18. Sachs, Paula, and Schmidinger, Ludwig: Über die chemische Grundlage der Ehrlichschen Diazoreaktion im Urin. IV. Mitteilung. Stufenphotometrische, quantitative Bestimmung des "blauen Farbstoffes," Ztschr. f. klin. Med. **132**: 73, 1937.
19. Sanford, A. H., Sheard, Charles, and Osterberg, A. E.: The Photelometer and Its Use in the Clinical Laboratory, Am. J. Clin. Path. **3**: 405, 1933.
20. Thierfelder, Hans: Handbuch der physiologisch- und pathologisch- chemischen Analyse für Ärzte und Studierende, Berlin, 1924, Julius Springer, 1004 pp.
21. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods. A Working Manual of Clinical Pathology, ed. 9, Philadelphia, 1939, W. B. Saunders Co., 841 pp.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

GRANULOMAS, Venereal and Non-Venereal of the Vulva, von Haam, E. J. A. M. A. **114:** 291, 1940.

The macroscopic and microscopic pictures of 155 cases of infectious granulomas of the vulva were observed in the laboratory for venereal diseases in New Orleans.

Because of the fundamental difference in diagnosis and therapeusis, a subdivision of infectious granulomas into the groups of venereal and nonvenereal lesions is recommended.

The presence of several etiologic factors in one pudendal granuloma could be proved in 11 cases. Various tests for the correct diagnosis of granulomas of mixed etiology have been evaluated.

Proper treatment of a granuloma of the vulva should be deferred until a complete diagnosis of the lesion has been reached. This will be greatly facilitated by the liberal use of laboratory methods.

THYROID DISEASE, A New Diagnostic (Galactose) Test for, Althausen, T. L., Lockhart, J. C., and Soley, M. H. Am. J. M. Sc. **119:** 342, 1940.

A new clinical test for activity of the thyroid gland based on the rate of intestinal absorption of galactose is described. It consists of oral administration of galactose followed by determinations of galactose in the blood thirty and sixty minutes later.

Data from 121 control subjects and 130 patients with hyperthyroidism show that the average maximal concentration of galactose in the blood of patients with hyperthyroidism was three times greater than normal and that clinically the test was comparable in reliability to estimations of the basal metabolic rate.

Following thyroidectomy, the galactose tolerance test was normal in almost all cases.

Advantages of the galactose test are that it is more sensitive than the basal metabolic rate in cases of low-grade hyperthyroidism, and that its outcome is not influenced by hyperventilation in anxiety states or by cardiac dyspnea. A disadvantage of the test is that the presence of hepatic insufficiency or of Paget's disease interferes with its use for the diagnosis of thyroid disease.

In myxedema abnormally low galactose tolerance curves were observed, indicating that the galactose test can be used also in the diagnosis of this condition.

The technique of the test follows:

Forty grams of galactose (Pfanstiehl) dissolved in 400 c.c. of water and flavored with lemon juice were administered by mouth after the patient had fasted overnight. Specimens of blood were obtained from the cubital vein before, and 5, 15, 30, 60, and 120 minutes* after administration of galactose.

The glucose fraction of the blood was removed by fermentation with ordinary yeast, according to the method of Somogyi as modified by Raymond and Blanco.

Preparation of Yeast.—A weighed amount of fresh commercial yeast (Fleischmann's) is suspended in 5 to 10 parts of water, centrifuged, and decanted. This is repeated until the supernatant liquid is clear and colorless and gives no reduction test (6 to 7 washings). The yeast is then suspended in 10 parts of water. In this condition it will keep in a refrigerator for about two weeks.

Use of the Hagedorn-Jensen Method.—Two-tenths cubic centimeter of blood is collected into an accurately calibrated pipette (the authors use 0.2 ml. in 0.001 ml. Kahn pipette

*At present the authors consider the 15- and 120-minute specimens unnecessary, and the 5-minute specimen relatively unimportant, except when the test is repeated on account of borderline results.

No. 37036), and transferred to 2.3 c.c. of distilled water, rinsing the pipette once or twice with the solution. One cubic centimeter of the 10 per cent yeast suspension is then added, the contents are mixed by tapping the tube against the hand, and after four or five minutes 0.5 c.c. of tungstic acid solution (prepared freshly by mixing equal volumes of 10 per cent sodium tungstate and ⅔ N sulfuric acid) is added. The test tube is covered with the thumb, and the contents are mixed quickly by inverting. After standing a few minutes, the mixture is centrifuged at high speed. One cubic centimeter of the clear filtrate is used for titration.

Use of the Folin-Wu Method.—The yeast is washed in the usual manner and a 20 per cent yeast suspension is prepared. To 2 c.c. of oxalated blood 14 c.c. of the yeast suspension and 4 c.c. of the tungstic acid solution are added. This mixture is filtered, and 2 c.c. portions of the filtrate are used for sugar determinations.

The figure for nonfermentable reducing substances in the fasting blood is subtracted from the corresponding figure in the remaining specimens to obtain the galactose content of the blood titrated as glucose. In order to obtain the true value for galactose, which has a lower reducing power than glucose, 24 per cent must be added to the last figure.

Galactose is used in this test because, while having a rate of absorption very similar to that of glucose, it has the advantage that, unlike glucose, it can be identified in the blood.

HEMATURIA, Diagnostic Value of Occult, Barach, J. H., and Pennock, L. L. J. A. M. A. **114:** 640, 1940.

Orthotolidine is a sensitive test for occult hematuria. A positive orthotolidine reaction was found in one-third of 681 general medical cases of the so-called chronic diseases. It is less common in youth, with more intact vessels, and it is more common in females than in males on account of the greater source of bleeding in the female generative tract. Seasonal variation pointed to the highest incidence during the summer months. Albuminuria and occult blood are found independently of each other, each having its own significance. Glycosuria is not a cause of occult bleeding. Arsenicals in therapeutic doses do not cause occult bleeding. Alkalinity or acidity of the urine is not a cause of occult bleeding. In patients showing persistent occult hematuria, hypochromic anemia is common. A review of the type of case in which the strongest reactions occurred reveals that these reactions were most pronounced in the clinically recognized serious types of disease and that the test reflects the patient's actual condition.

STREPTOCOCCI, Human Hemolytic From Diseases of Children, Boisvert, P. L. Am. J. Dis. Child. **59:** 281, 1940.

The Lancefield group precipitin test gave consistent results and seems well adapted for epidemiologic use. The results suggest that infants and children are no more, and may be less, susceptible than adults to infection by hemolytic streptococci or groups other than A. It seems significant to the author that during a period of over a year all the strains of hemolytic streptococcic infections and their possible sequelae should belong in the one group, group A. Thus, 509 strains of group A hemolytic streptococci were consecutively obtained from 262 pediatric patients.

Infants and children are no more susceptible than adults to infection by hemolytic streptococci of groups other than A.

This study is being continued and is now in its fourth year. The only exception to date is the isolation this fall of a group F hemolytic streptococcus from the blood of a 7-year-old child with a nonfatal septicemia.

PERICARDIUM, Milk Spots, Nelson, A. A. Arch. Path. **29:** 256, 1940.

Pericardial milk spots occurred in 170 (24.4 per cent) of 494 persons 1 year or more of age. In 439 persons 18 or more years of age the incidence was 37.6 per cent. In general, there is an increase of incidence with age, but this increase is by no means rectilinear. The spots are scarce in children and very frequent in old age, but between 35 and 75 years of age there is little change in incidence.

There seems to be a definite association with chronic or recurrent valvular heart disease; of 42 persons with such disease, 28 (66.7 per cent) showed spots. Patients with severe coronary sclerosis and enlarged hearts showed fairly definite increases (50.0 and 47.9 per cent, respectively).

The occurrence of more than one spot is slightly more frequent than that of only one. Spots occur on the right side, anteriorly, and on the ventricles much more frequently than they do on the opposite surfaces.

Old pleural adhesions are slightly more frequent in patients with spots than in those without.

Fifteen or 20 per cent of spots show appearances (projecting villi, cellular exudation, subepithelial, palisading, or epithelial enclosures in collagenous tissue) which suggest transitions from a more active inflammatory process to the usual type of milk spot.

SULFAPYRIDINE THERAPY, Nature of the Renal Lesion With, Stryker, W. A. J. A. M. A. **114**: 953, 1940.

Precipitation of acetylsulfapyridine in the kidney was found at autopsy of a patient after treatment with sulfapyridine. The marked dilatation of cortical tubules and glomerular spaces, characteristic of kidneys in cases of sulfapyridine toxicity, seems to depend on intratubular precipitation of the drug or its compounds. There is a possible explanation for the previous difficulty in demonstrating the material in histologic sections.

DEXTROSE TOLERANCE, Tests of the New Born, Ketteringham, R. C. Am. J. Dis. Child. **59**: 542, 1940.

By the Jeghers-Myers modification of the Folin-Malmros ferricyanide micromethod, the blood sugar values of 16 normal newborn infants, 5 to 10 days old, ranged from 70 to 95 mg. per hundred cubic centimeters three and one-half to four hours after feeding, with a mean of 81 mg.

Dextrose tolerance tests were made on 15 normal infants. Oral administration of 1.75 Gm. of dextrose per kilogram of body weight, as a 10 per cent solution, with blood sugar determinations one-half, one, two, three, and four hours after the midpoint of the period necessary for ingestion, produced an average maximum level of 143 mg. per hundred cubic centimeters. Two distinct curves were present; the blood sugar value of formula-fed infants reached a mean maximum of 143.2 mg. at thirty minutes, while that of breast-fed infants reached a mean maximum of 142.6 mg. at one hour.

Response to the dextrose meal was higher than in previously reported work; apparently these infants responded just as young adults do. The hypoglycemic reaction of Foster was encountered in 11 of the 14 babies tested.

Infants of diabetic mothers were more difficult to test than were the controls, and the curves produced seemed less reliable than those of the controls, owing to technical factors which could not be regulated. One infant who had previously showed the clinical signs of hypoglycemia had a strikingly erratic curve.

The Exton-Rose one hour, two-dose test, modified to the dose-weight principle, was administered to 3 normal infants, but the nature of the divided dose leads one to believe this technique unsuitable for the newborn.

GONORRHEA, Culture Method in the Diagnosis of, A New Medium, Pitts, A. C. Ven. Dis. Inform. **21**: 67, 1940.

A relatively simple medium for the culturing of gonococci has been described.

This medium is a variation of the Schwartz-Davis medium and consists of a testicular infusion agar to which sterile hydrocele, ascitic, or pleural fluid is added. It has proved a most satisfactory medium for primary cultures.

The preparation of the medium follows:

Infuse 500 Gm. of minced sheep testicles in 1 liter of distilled water overnight in the icebox. (The testicles should be from recently killed animals.) Strain through gauze, boil vigorously for one-half hour, and strain through gauze again. Restore volume with distilled water and boil another one-half hour to complete coagulation, strain through gauze and

filter through paper. If coagulation is complete, filtration will be relatively easy by the use of suction; if coagulation is not complete, it is advisable to boil the infusion a little longer. Restore volume and add:

20 Gm. bacto-peptone
5 Gm. sodium chloride
5 Gm. dextrose

Adjust the reaction to pH 7.8 (pH should be between 7.4 and 7.6 after autoclaving) and add:

20 Gm. bacto-agar

Heat until agar is melted (autoclaving the mixture twenty minutes is a satisfactory method), filter through gauze and cotton, and tube approximately 5 c.c. per tube. Autoclave for thirty minutes at 10 pounds' pressure. When cooled to 50° C., add approximately 2 c.c. of sterile hydrocele fluid, rotate the tube to insure an even mixture, and slant. As soon as the agar is hardened, replace the cotton stoppers with rubber ones; it is important to do this as soon as possible in order to retain a maximum amount of moisture in the tubes. This medium will keep in the incubator for several months.

UREA, Rapid Methods for the Determination of, in Blood and Urine, Scott, L. D. Brit. J. Exper. Path. **21**: 93, 1940.

1. Centrifuge tubes graduated at 5 c.c., and thick-walled test tubes of approximately 6 by 5/8 inches.
2. Pipettes of 0.2 c.c., 1 c.c., 2.5 c.c., and 3.5 c.c. capacity.
3. Two 5 c.c. microburettes graduated to 0.05 c.c.
4. N/20 and N/100 sulfuric acid.
5. Approximately N/50 sodium hydroxide.
6. No. 1 Whatman filter papers of 5.5 cm. diameter.
7. Absolute alcohol and redistilled or "Analar" acetone.
8. Special mixed indicator:

Stock Solution A: 0.02 per cent methyl red in 60 per cent (by volume) absolute alcohol. The solid methyl red should be ground up with absolute alcohol in a small mortar till completely dissolved, and transferred to the 100 c.c. flask by repeated washings with absolute alcohol in the usual manner. Forty cubic centimeters of water are added and the volume is brought to 100 c.c. with absolute alcohol.

Stock Solution B: 0.10 per cent aqueous methylene blue.

The indicator is prepared by adding 1 c.c. of the methylene blue (solution B) to 15 c.c. of methyl red (solution A). The mixture, if green in color, is brought to a very faintly acid reaction by adding N/10 hydrochloric acid drop by drop with constant shaking until the solution just turns reddish purple. This solution keeps indefinitely if stored in an amber ground glass-stoppered bottle. It is reddish purple in acid solution, and bright green in alkaline solutions.

9. Neutral "Urease-Dunning" enzyme suspension:

Four 25 mg. tablets of "Urease-Dunning" are thoroughly ground up in a small mortar with 4.5 c.c. of N/100 sulfuric acid. When an even emulsion has been obtained, 0.2 c.c. of the mixed indicator is added. The extract, if correctly prepared, has a grayish buff coloration (the neutral point of the indicator) and keeps in the refrigerator for one month.

Determination of Urea in Blood.—Into one of the 5 c.c. graduated centrifuge tubes, containing 3 to 4 c.c. of absolute alcohol or acetone, pipette 1 c.c. of serum, oxalated whole blood, or plasma. As it is impossible to remove the last traces of blood from the pipette by sucking up and blowing out with alcohol in the usual manner (due to the pipette becoming clogged with precipitated blood protein), the pipette must be allowed to drain thoroughly for about one minute, and the last traces of blood removed by expelling the air. By this procedure not more than 0.01 c.c. of blood is retained in the pipette. The centrifuge tube is now securely stoppered and vigorously shaken, after which the volume is readjusted to 5 c.c. by the further addition of a few drops of absolute alcohol or acetone, as the case may be, to counteract the reduction in volume due to the formation of alcoholic hydrates. Mix again by shaking well and centrifuge at high speed for two to three minutes, or filter

through a No. 1, 5.5 cm. paper. After filtration the precipitated blood protein contained in the filter paper should be disturbed by very gentle stirring with a small glass rod, otherwise the 2.5 c.c. of filtrate may not always be obtained from bloods rich in metaprotein. Measure 2.5 c.c. of supernatant fluid or filtrate into one of the thick-walled test tubes and add 3.5 c.c. of water. The alcoholic extract becomes slightly opalescent due to the partial precipitation of blood fat by the lowered alcoholic concentration. Three-tenths cubic centimeter of special indicator is added and the alkaline reaction (green) brought over to the acid side (reddish purple) by the addition of N/20 sulfuric acid, with thorough mixing after the addition of each drop. The alcoholic extract varies in alkalinity according to the blood bicarbonate content. Fiftieth-normal sodium hydroxide is carefully added until the reaction is just alkaline again, and the reaction finally brought over to the very faintly acid point of the indicator by the addition of N/100 sulfuric acid, drop by drop, with constant shaking. By this readjustment of the pH, final titration with relatively strong acid is avoided, enabling the starting point of the reaction to be controlled to within one drop of N/100 sulfuric acid, which in terms of blood urea is approximately 1 mg. per 100 c.c. Finally, 0.35 c.c. of well-shaken neutral urease suspension is added, and after thorough mixing, the tube is securely stoppered and heated in the water bath at 50° to 55° C. for ten to fifteen minutes. Remove the tube from the bath and titrate with N/100 sulfuric acid until slightly acid (the original reddish purple starting point of the reaction). The titration value in cubic centimeters multiplied by 60 equals milligrams of urea per 100 c.c. of blood.

Determination of Urea in Urine.—For the estimation of urea in urine a 1:50 dilution of the specimen is made with distilled water (with small quantities of urine 0.2 c.c. can be measured with a blood pipette into 9.8 c.c. of water) and after mixing well, 5 c.c. is pipetted into a thick-walled test tube and 0.3 c.c. of indicator is added. The faint greenish coloration obtained with the majority of urines is due to the urine being alkaline to the special indicator. Adjustment is made to the faintly acid side of the indicator by the addition of N/100 sulfuric acid drop by drop. One-half cubic centimeter of well-shaken neutral urease suspension is now added, the tube is securely stoppered, and the mixture (which turns green almost immediately) is heated in the water bath at 50° to 55° C. for five minutes. The ammonium carbonate so formed is back titrated with N/20 sulfuric acid till just reddish purple, i.e., the original starting point of the reaction. The titration value in cubic centimeters multiplied by 1.5 equals grams of urea per 100 c.c. of urine.

BLOOD, A Familial Hematopoietic Disorder in Italian Adolescents and Adults, Wintrobe, M. M., Matthews, E., Pollack, R., and Dobyns, B. M. J. A. M. A. **114**: 1530, 1940.

Fourteen individuals, members of three Italian families representing three generations in one family and two in another, have been examined and have been found to have certain abnormalities.

In four marked poikilocytosis, microcytosis, hypochromia, and stippling of the red blood cells as well as corpuscles resembling targets were observed. In addition, there were increased resistance of the red corpuscles to the hemolytic action of hypotonic saline solutions, splenomegaly, and bilirubinemia or urobilinuria. One of these patients also had erythroblastosis in the sternal bone marrow and slight osteoporosis.

In four persons all the abnormalities mentioned except splenomegaly were found. In two children there was slight splenic enlargement without significant changes in the blood. Of the remaining four persons, all females, there was microcytosis without other significant changes in two, and slight macrocytosis as well as stippling in two.

This disorder may be considered to be a benign form of Cooley's anemia or Mediterranean disease.

This condition and Cooley's anemia appear to be the result of an inherited defect in the production of red corpuscles whereby corpuscles are formed with an adequate or excessive membrane which contains little substance. As a result the cells can withstand the hemolyzing effect of hypotonic saline solutions more readily than normal cells. The other characteristics of the disorder are explained by the assumption that they are due to attempts to compensate for the faulty red blood cell formation.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Fundamentals of Internal Medicine*

DESIGNED primarily for the introduction of students to the study of internal medicine, this volume, nevertheless, contains good chapters dealing with otolaryngology, ophthalmology, and dermatology. Because photographs of dermatoses show up better in white persons than in Negroes, it is felt that the section on dermatology would be improved by changing such illustrations. A chapter on dietetics is also provided.

The work is intended to provide the foundation upon which the superstructure of more detailed and extensive knowledge may be built. In this aim it succeeds admirably.

Cancer†

THIS volume was prepared by a committee representing the Massachusetts Medical Society and the American Society for the Control of Cancer. It is intended for the general practitioner and is designed to aid him in the diagnosis of early cancer and to suggest the treatment recognized at the present time. Chapters on cancer of the various organs are written by men especially interested in the subject.

The discussion of the care of the patient with advanced cancer, and neurologic methods of relief of pain are also handled in an able manner.

The various organizations for the control of cancer, local and national, are described. There is a concluding chapter on suggestions for talks on cancer to lay audiences.

The book is brief and is not intended to take the place of the more extensive treatises in the field.

It is felt that this is excellent propaganda, using the word in its best sense.

Manson's Tropical Diseases‡

PROBABLY the outstanding text in its field, this book has now come into the eleventh edition. Although the term "tropical medicine" is in general usage, it is perhaps a misnomer. One has only to recall the Chicago outbreak of amebiasis, the cases of malaria in

---

*The Fundamentals of Internal Medicine. By Wallace Mason Yater, A.B., M.D., M.S. (in Medicine), Professor of Medicine and Director of the Department of Medicine, Georgetown University School of Medicine; Physician-in-chief, Georgetown University Hospital; Physician-in-chief, Gallinger Municipal Hospital, Washington, D. C., Formerly Fellow in Medicine, the Mayo Foundation, Rochester, Minn. Cloth, 1,021 pages. D. Appleton-Century Company, Inc. New York, N. Y., and London.

†Cancer—A Manual For Practitioners. The Committee on Publication: George W. Holmes, M.D., Chairman; Ernest M. Daland, M.D.; Shields Warren, M.D.; and Channing C. Simmons, M.D., Editor. Cloth, 284 pages. Boston, Mass., 1940.

‡Manson's Tropical Diseases. A Manual of the Diseases of Warm Climates. Edited by Philip H. Manson-Bahr, C.M.G., D.S.O., M.A., M.D., D.T.M.; and H. Cantab, F.R.C.P. Lond., Senior Physician to the Hospital for Tropical Diseases, London, the Albert Dock Hospital and the Tilbury Hospital; Consulting Physician to the Colonial Office and Crown Agents for the Colonies; Consultant in Tropical Diseases to the Royal Air Force; Director, Division of Clinical Medicine, London School of Hygiene and Tropical Medicine; Lecturer on Tropical Medicine to the London Hospital, the West London Post-Graduate College, and the North-Eastern Post-Graduate College; Corresponding Member of the Societe de Pathologie Exotique; Member of the Washington Academy of Medicine; late Examiner in Tropical Medicine to the Conjoint Board of the Royal College of Physicians and Royal College of Surgeons, England, and to Cambridge and Hongkong Universities; Author (with A. Alcock) of "The Life and Work of Sir Patrick Manson," 1927; and "The Dysenteric Disorders," 1939. Cloth, ed. 11, revised, 1,083 pages, 18 color plates, 15 halftone plates, 364 illustrations in text, 6 maps, and 28 charts, $11.00. A William Wood & Co. book. Williams & Wilkins Co., Baltimore, Md., 1940.

narcotic addicts, the widespread incidence of intestinal parasites, and the great amount of bacillary dysentery, to realize that we have many "tropical" diseases in this country. In addition to a standard description of these and many other diseases, the book contains a section on life in the tropics of great value to anyone contemplating a trip or an extended sojourn into tropical climates. Anyone called upon to give advice to such persons will not be disappointed if he seeks information at this source.

Clinical Diabetes Mellitus and Hyperinsulinism*

IN THIS easily readable book the author has limited himself to clinical considerations describing procedure which in his extensive experience has proved most effective in the recognition and treatment of diabetes mellitus and its complications. Extended consideration of the theory of carbohydrate metabolism, the physiology of experimental diabetes, and the pathology of diabetes are omitted.

Description of techniques of diagnosis, diet, and insulin (old and new) is given in sufficient, but not excessive, detail. Much attention is directed toward the ills which so often are concomitant. Chapters are devoted to surgical operations and to pregnancy in diabetes.

A thorough section is afforded to hyperinsulinism in the last part of the book. There is a good bibliography and index.

Principles of Hematology†

IT IS pointed out that the symptoms of blood disorders do not help greatly in differentiation. An anemia from any cause will result in weakness, pallor, and palpitation of the heart. There may be fever, anorexia, dizziness, and edema. Few of the anemias have characteristic accompanying symptoms, with the striking exception of pernicious anemia. There are few characteristic symptoms of leucemia. The disease may be suspected from a splenomegaly, enlarged glands, fever, unexplained anemia, or abnormal bleeding. A careful history may reveal the explanation of an anemia, such as a previous blood loss or an acute or chronic infection. A thorough physical examination may reveal a malignancy.

Written by an outstanding hematologist, the book is well balanced. The anatomy and physiology of the blood, the mechanisms involved in the various dyscrasias, the techniques of blood examination, and finally treatment, are all excellently integrated.

---

*Clinical Diabetes Mellitus and Hyperinsulinism. By Russell M. Wilder, M.D., Ph.D., F.A.C.P., Professor and Chief of the Department of Medicine, the Mayo Foundation for Medical Education and Research, University of Minnesota; Head of the Section on Metabolism Therapy, Division of Medicine, the Mayo Clinic, Rochester, Minn. Cloth, 459 pages, illustrated. W. B. Saunders Co. Philadelphia and London, 1940.

†Principles of Hematology. By Russell L. Haden, M.A., M.D., Chief of the Medical Division of the Cleveland Clinic, Cleveland, Ohio; Formerly Professor of Experimental Medicine in the University of Kansas School of Medicine, Kansas City, Kan. Cloth, ed. 2, thoroughly revised, 362 pages, with 104 illustrative cases and 167 illustrations, including 173 original photomicrographs and 100 original charts and drawings. Lea & Febiger, Philadelphia, 1940.

EDITORIAL

Clinical Chemistry

A NEW section on Clinical Chemistry is being inaugurated with this issue of the JOURNAL. This does not mean that the original scope of the JOURNAL, as defined by its first Editor, Dr. Victor C. Vaughan,[1] in the first issue, has in any way been changed. In the quarter of a century that has elapsed, many important advances in medicine have been made, and chemistry has contributed a large number of them. This has resulted in a considerable increase in the number of papers which have been devoted to studies falling under the classification of clinical chemistry. For this reason, it now seems desirable to group these articles together under this special heading.

Dr. Vaughan unquestionably visualized the expansion in this field when he founded the JOURNAL, since he was a pioneer* in this country in the field of biochemistry and clinical chemistry—then called physiological chemistry and pathological chemistry, respectively. It is pertinent to note that the subject of blood chemistry was in its infancy in 1915 when this JOURNAL was founded. The expansion which has taken place in this field is typical of the expansion which has occurred in many other fields of biochemistry. Although the accomplishments in blood chemistry are probably no more spectacular or important than those which have taken place in the field of the vitamins or of the hormones, still blood chemistry requires much greater use of the clinical laboratory and has more far-reaching ramifications. The latter is well illustrated by the fact that the action of vitamin D is shown by studies of the inorganic phosphorus and calcium of the blood serum, while that of insulin requires the determination of the blood sugar.

Recently the methods of chemical blood analysis have been extended to tissue analysis; this will doubtless greatly expand the interpretations which can be made upon the basis of blood analysis alone. By utilizing some of these micromethods after still further refining them, Richards,[2] in particular, has been able to develop a sound theory of urinary secretion, thus placing our chemical tests of renal function upon a firmer foundation. Illustrative contributions in the field of clinical chemistry need not be further multiplied.

While this JOURNAL has had a hand in presenting some of the most important original contributions—for example, Banting and Best[3] presented their initial report on insulin here, and Stanley Benedict[4] reported the excretion of uric acid by the Dalmatian coach dog, thus permitting the extension of studies on purine metabolism previously restricted to man (and the anthropoid apes)—still its greatest service to medicine has been in bringing new practical tests to the clinical laboratory and in turn to the clinic. This is in accord with the title of the JOURNAL and Dr. Vaughan's first editorial,[1] which indicated that the

*Dr. Vaughan was appointed Instructor in Physiological Chemistry at the University of Michigan in 1876.

purpose of the JOURNAL was to try to bridge the gap between the laboratory worker and the clinic, so that the physician could intelligently utilize without unnecessary delay the new laboratory aids which were rapidly becoming available. This need is even greater today than it was twenty-five years ago.

Clinical chemistry may be defined as that part of biochemistry which finds use in the diagnosis, prognosis, and clinical management of disease. The JOURNAL will accept for the section on clinical chemistry original articles, including methods, which meet a reasonably broad interpretation of this specification. It is hoped that this new section will be found increasingly useful by internists and clinical chemists alike.

V. C. M.
E. M.

REFERENCES

1. Vaughan, V. C.: The New Journal, J. LAB. & CLIN. MED. **1**: 57, 1915.
2. Richards, A. N.: Physiology of the Kidney, Bull. New York Acad. Med. **14**: 5, 1938.
3. Banting, F. G., and Best, C. H.: The Internal Secretion of the Pancreas, J. LAB. & CLIN. MED. **7**: 251, 1922.
4. Benedict, S. R.: Uric Acid in Its Relation to Metabolism, J. LAB. & CLIN. MED. **2**: 1, 1916.

Erratum

On page 1301 of the September number of the JOURNAL in the article by Ida Kraus, Ph.D., entitled "A Note on the Determination of Total Serum Proteins, Serum Albumin, and Serum Globulin," *c* under *Calculations* should read:

$$\text{N.P.N.} : \frac{S}{U} \times \text{Conc. standard} \times \frac{15}{4.5} \times \frac{100}{1} = \text{N.P.N. / 100 c.c. serum.}$$